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MUSCULAR EFFICIENCY ON HIGH CARBOHYDRATE AND HIGH FAT DIETS

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ZUNTZ (1896) was unable to substantiate the hypothesis of Chauveau which held that the body could use only carbohydrate as a source of energy and that there was an energy wastage of 30 per cent, on account of its transformation into carbohydrate, when fat was the source. When animals or men were made to do work on a fat diet, he found practically the same energy was expended as when the work was done at the expense of carbohydrate. Krogh and Lindhard (1920) sought to settle the old dispute as to the efficiency of the body on the two main sources of energy, and concluded that the body was some 10 or 11 per cent relatively more efficient upon carbohydrate than upon fat. This value has been accepted by all workers and by some (Hill, 1924) has even been used as one of the main arguments that the muscles can get their energy only by the breakdown of carbohydrate and that fat is used only by first being transformed into carbohydrate; although the authors themselves say "It is clear that our experiments cannot be used as evidence to prove that the fat must be converted into sugar before being used in muscular work."

Krogh and Lindhard did not measure the protein metabolism and while it is generally conceded that protein metabolism is not changed materially by work, yet the review of the literature and of his own results published recently by Cathcart (1925) seem to indicate that for long-continued, or heavy work, at least, there is a small though definite increase in nitrogen excretion. There is a possibility that on a diet consisting almost exclusively of fat there would be increased protein metabolism over that found on a high carbohydrate diet, since the latter group of substances are known to be much more effective protein sparers than are fats. The latter fact has been abundantly demonstrated by many workers, and quite con-

clusively by Landergren (1903) who reduced the nitrogen excretion in man to about 4 grams by giving diets containing chiefly carbohydrate and about one gram of nitrogen, but when fat entirely replaced carbohydrate the nitrogen excretion rose to the starvation level, about 10 grams a day. So long as abundance of carbohydrate remains, however, the replacement of a considerable portion of this foodstuff by fat does not increase but may further decrease protein metabolism, as shown recently in a prolonged experiment on the nitrogen minimum by Smith (1926).

If there should be marked increase of protein metabolism during work on a high fat diet, it would result in an increased heat production which would appear as a result of the work, but which in reality would be a specific dynamic effect of the protein. Or, if there should be a difference in the rate of fall of nitrogen excretion several hours after a meal when work is done on high fat as compared with high carbohydrate, a different distribution of the calculated heat to the several foodstuffs would result, which might affect the total heat and therefore the calculated efficiency. Since the extra heat produced, resulting from the specific dynamic action of protein, cannot be used for work (Rubner, 1910, Anderson and Lusk, 1917) there is always summation of this factor with the extra heat of the work itself. An increased protein metabolism therefore might conceivably account for part, at least, of the lower efficiency noted by Krogh and Lindhard on the high fat. It seemed a point worthy of further investigation although at least one report in the literature seems to show exactly the opposite result. Heineman (1901) reported experiments made on man working with a Gärtner ergostat in which, on protein-rich diet and likewise on carbohydrate-rich diet, an increased N output was obtained during work, while on a fat-rich diet there was an apparent decrease.

The experiments to be reported in this paper were done on a single subject, B.C., who was a young boxing instructor, in good training generally although not specifically trained for bicycle riding. The experiments have already been reported in a previous paper (Marsh, 1928) so far as the effect of work on the character of the metabolism is concerned. This subject was first studied on a normal diet and the efficiency calculated in the usual manner. He was then placed on a high carbohydrate diet, later on high fat, a second time on high carbohydrate and finally again on high fat. The diets used have been described in detail in the previous paper. We summarize below merely the distribution of energy for the two diets.

All of the experiments on this subject were made with the Krogh bicycle ergometer, very kindly loaned to us by Prof. Graham Lusk. As is well

known the work is calculated upon the basis of the amperage of the electric current passed through the magnetic brake. Calculation of the heat production is based upon the caloric values of oxygen as originally worked out by Zuntz and Schumburg (1901) and as more recently modified by Lusk (1924), using the non-protein R.Q. and upon the heat value of the protein metabolism.

APPROXIMATE CALORIC VALUE OF DIETS

Diet	Total Cal.	Cal. from Fat.	Cal. from Carboh.	Cal. from Prot.
Carbohydrate	2400	180	1920	300
Fat	2800	2200	340	260

Since urine cannot be collected quantitatively from human subjects in much less than one-hour periods it was not possible to secure clear-cut separation of the resting protein metabolism from work metabolism, and for the following additional reason. The first or control period of N excretion was for about one hour and included the pre-work rest period. The second N period of about the same length began just before the work started, but, because, for purposes of controlling recovery on O_2 and CO_2, the post-work rest period followed very closely the work and recovery period, it included the post-work rest period as well as work and recovery. As we shall see, it is rather striking that the protein metabolism of this second period is usually less than in the previous period especially in the post-absorptive condition.

The net efficiency is found by subtracting from the total metabolism of the work and recovery period, the metabolism as obtained in the pre-work resting period, during which the subject sat quietly on the ergometer, but calculated to a length of period equal to that of work and recovery together, and dividing this difference into the heat equivalent for the work done.

Tables I to V give the results of these experiments on normal and on special diets, showing the oxygen absorption, non-protein R.Q., nitrogen elimination per hour, and the net efficiency. On the normal diet which was not controlled (Table I) the work was varied from 4.5 Cals. to 13.7 Cals. total, by changing the speed and duration of work. There is no close relationship in these individual experiments between the total amount of work done and the efficiency. The average efficiency in seven experiments in which the work averaged 6.80 Cals. is 22.4 per cent while for five experiments in which the work was nearly twice as much, namely,

TABLE I

METABOLISM AND NET EFFICIENCY—NORMAL DIET

Date 1927	Exp. No.	Day of Diet	Work Cal. Per Min.	Total O_2 cc. per min.		Non-Prot. R.Q.		N. Excretion mgm. per Hr.		Excess Cal.	Total Work Cal.	Net Eff. %	Hrs. aft. breakfast or post absorptive
				Pre Work	Work & Recov.	Pre Work	Work & Recov.	Pre Work	Work & Recov.				
2–15	11[1]	7	1.38	249	604	0.78	0.87	506	369	29.96	6.92	23.1	3½ hrs.
2–16	12	8	1.35	232	623	0.83	0.91	360	312	29.28	6.67	22.8	4½ hrs.
2–17	13	9	.90	216	551	0.80	0.81	440	385	21.01	4.52	21.5	Post Abs.
2–17	14	9	1.37	236	632	0.79	0.88	300	360	29.41	6.85	23.3	5½ hrs.
2–18	15	10	1.36	221	649	0.79	0.89	373	307	31.91	6.82	21.4	Post Abs.
2–18	16	10	—	264	657	no analysis							
2–19	17	11	1.37	216	687	0.84	0.90	362	232	63.12	13.70	21.7	Post Abs.
2–21	18	13	1.37	219	712	0.86	0.95	543	393	69.52	13.70	19.7	Post Abs.
2–22	19	14	1.50	219	624	0.87	1.00	356	283	35.35	7.52	21.3	Post Abs.
2–22	20	14	1.39	261	671	0.86	0.93	372	404	34.88	8.36	24.0	4½ hrs.
2–24	21	15	1.38	239	662	0.80	0.92	444	297	49.00	11.10	22.7	Post Abs.
2–25	22	16	1.38	229	643	0.73	0.88	302	287	47.52	11.10	23.4	Post Abs.
2–26	24	17	1.40	231	677	0.76	0.88	409	512	50.80	11.20	22.0	Post Abs.
Average of last 7 experiments			1.39	231	668	0.82	0.92	398	344	50.04	10.95	22.1	

[1] Experiments not included in the tables were determinations of the R.Q.'s only, during various stages of work and recovery.

12.16 Cals. the efficiency is 21.9 per cent. There is also little difference in efficiency when these experiments are arranged according to Cals. of work per minute. The highest rate was 1.5 Cals. per minute with an efficiency of 21.3 per cent and the lowest was 0.9 Cals. per minute with an efficiency of 21.5 per cent. However, several experiments at a higher rate show a higher efficiency than this last figure. The last seven experiments, because they were done at the same rate of work per minute as those on the special diets, form the basis for a normal average.

Apparently the variation in efficiency found in this series (of seven) with a standard deviation of 1.13 per cent (absolute) and a coefficient of variability of 5.0 per cent (relative), are not due to differences in total work done or in rate of work, and we are justified in taking this average of 22.1 per cent as a reliable basis for this subject within the range of work mentioned, so long as he was on a normal diet, with which to compare the values obtained on the special diets.

When the subject was placed on the special diets he ate two meals a day at the laboratory, the food each time being carefully weighed. He came to the laboratory in the morning without breakfast, ran an experiment, and had his first meal between 10 and 11:00 o'clock. Another run was made in the middle or late afternoon, followed by dinner at 5:00 o'clock. The subject then took home with him a weighed amount of food for a lunch later in the evening. This arrangement made it possible to have one experiment in the morning in the post-absorptive state and a second four or five hours after a meal. The efficiencies throughout are calculated as net, the heat production in the pre-work period being used as a base line. A post-work period was also run for the sake of checking recovery, which has been fully discussed in the previous paper, but this period did not enter into the calculation of efficiency except that it was included in the work period for the nitrogen excretion.

First High Carbohydrate Diet

In Table II are found the results of eight experiments on the special carbohydrate diet. As compared with the normal series the non-protein R.Q. is higher both in the initial period and in the work and recovery period, thus showing plainly that the special diet supplied more carbohydrate than the subject was accustomed to take when he was permitted an entirely free choice of foods. No attempt was made to "stuff" carbohydrate so as to make the quotients as high as possible. The strenuous activity of the subject outside the laboratory also probably contributed to keep the quotients down. He was feeling fine on the special diet and

TABLE II

METABOLISM AND NET EFFICIENCY—CARBOHYDRATE DIET, FIRST SERIES

Date 1927	Exp. No.	Day of Diet	Work Cal. Per Min.	Total O_2 cc. per min.		Non-Prot. R.Q.		N. Excr. Mgm. per Hr.		Excess Cal.	Total Work Cal.	Net Eff. %	Hrs. aft. breakfast or post absorptive
				Pre Work	Work & Recov.	Pre Work	Work & Recov.	Pre Work	Work & Recov.				
2–28	25	2	1.40	244	601	0.77	0.96	575	441	45.88	11.23	24.5	Post Abs.
2–28	26	2	1.40	215	633	0.94	0.91	327	354	50.33	11.22	22.3	5 hrs.
3–1	27	3	1.40	237	636	0.73	0.91	568	457	49.38	11.22	22.7	Post Abs.
3–1	28	3	1.41	247	618	0.77	0.91	466	463	48.52	11.31	23.3	6 hrs.
3–3	29	5	1.39	204	611	0.87	0.97	624	391	51.71	11.16	21.6	Post Abs.
3–3	30	5	1.40	207	592	0.93	0.95	501	414	50.11	11.23	22.4	4 hrs.
3–4	31	6	1.39	207	611	0.86	0.89	430	221	52.03	11.15	21.4	Post Abs.
3–5	32	7	1.40	218	570	0.92	0.91	611	311	47.26	11.20	23.7	Post Abs.
Average			1.40	222	609	0.85	0.93	513	381	49.40	11.21	22.7	

several times boxed in the evening or danced, either of which form of activity probably used up a fair proportion of his glycogen supply and resulted in the low initial quotients recorded. However, as the series of experiments progressed the initial quotient came to a higher general level. The average increase in the non-protein work R.Q. over the initial R.Q. in the last seven experiments of the control series (Table I) was 0.10 (0.82 to 0.92) while in the special carbohydrate series it was 0.08 (0.85 to 0.93).

The protein metabolism of the normal series showed an average difference between the two periods (1st containing the initial rest and the 2nd containing the work-and-recovery *as well as* a rest period), of 54 milligrams of nitrogen per hour. The average difference on the special carbohydrate diet between the same two periods was 132 milligrams. In both series the excretion of nitrogen in the second period was lower than in the first. Part of this obviously is due to the steady decline in nitrogen excretion from hour to hour since the last protein meal. This would apply more especially to the post-absorptive experiments. There is only one exception in the normal series (Feb. 26, a day when the initial R.Q. was 0.76) and none at all on the special diet. In two experiments of the normal series (Nos. 14 and 20, Table I) when the experiment was run 5½ and 4½ hrs. respectively after breakfast the nitrogen excretion in the 2nd collection (work) period was higher than in the first. This is likewise true in one experiment (No. 26, Table II) on the high carbohydrate diet, and in one other (No. 28) the excretion is almost as high 6 hrs. after breakfast. The protein contained in the breakfast must have been the principal cause. With these exceptions in all the experiments (i.e. in 9 out of 12 in the normal series, and 7 out of 8 in the high carbohydrate series) the nitrogen excretion is significantly lower in the work period.

Why should it be 80 milligrams lower (average) on the special carbohydrate diet than on normal diet when the subject worked? It must be remembered that seven of the twelve experiments recorded on the normal diet and five of the eight on high carbohydrate were post-absorptive, i.e. at least twelve hours after last food. It is our belief that the average result reflects the sparing effect of carbohydrate mobilized by the effort of work from abundant glycogen stores. The high non-protein R.Q.'s support this conception. Also, as we shall see, the contrasting results of the following experiments on fat diets.

The net efficiency on the normal diet has already been mentioned (p. 110). More carbohydrate apparently has raised the average net efficiency from 22.1% to 22.7% (Table II). The standard deviation is 0.97% (absolute

instead of 1.13 and the coefficient of variability is 4.3% (relative) instead of 5.0. The special diet, because more uniform and especially because it was taken more regularly, made the results slightly more concordant than the normal diet. The work which amounted to 1.4 Cal. per minute for eight minutes was performed with comparative ease. There was marked perspiration but the subject was never tired out by the run (contrast the effect on high fat, below, and always remarked that he could have kept it up much longer.

First High Fat Diet

Immediately after the last experiment recorded in Table II, the high fat diet was started (March 5) and was continued for eleven days. This was much longer than was planned owing to trouble with the respiration machine which caused delays.

After the first two or three days the subject felt very tired and sleepy, in fact did sleep usually several hours between experiments, and had no desire to work or to do anything else. The work seemed harder, although actually it was the same as in the previous series. In the last few experiments the subject became dizzy on the ergometer toward the end of the work period and afterward expressed fear that he might have fallen off. But he stuck to it pluckily until the series was completed. The very last experiment had to be discontinued at the end of 6¾ minutes because of dizziness. These subjective symptoms were by no means imaginary for the subject had no previous knowledge of the probable effects of a fat diet and in fact when on the carbohydrate diet thought that he would be more efficient on fat because it would "stay by" him longer. It did to his sorrow "stay by" him longer but not in the way he anticipated and it did not make him more efficient.

The average net efficiency of the nine experiments recorded (Table III) is 21.5%, or 1.2% less than on high carbohydrate. Because of leaks in the machine there were no experiments on the first three days of the diet. Probably if results on these first few days had been obtained, the average would have been higher (see 2nd High Fat series). The average given excludes the last experiment which had to be interrupted before the end of the full eight minutes, and was probably invalidated also by an unusually high initial metabolism.

This is the first series which shows a marked difference in efficiency between the post-absorptive and post-prandial experiments. The average for the former is 21.0% and for the latter 22.5%. The subject felt no more like working after the meal and after a good nap than before. However,

TABLE III

METABOLISM AND NET EFFICIENCY—FAT DIET, FIRST SERIES

Date 1927	Exp. No.	Day of Diet	Work Cal. Per Min.	Total O_2 cc. per min.		Non-Prot. R.Q.		N. Excr Mgm. per Hr.		Excess Cal.	Total Work Cal.	Net Eff. %	Hrs. aft. breakfast or post absorptive
				Pre Work	Work & Recov.	Pre Work	Work & Recov.	Pre Work	Work & Recov.				
3–8[1]	34	4	1.39	217	595	0.79	0.83	651	446	51.43	11.18	21.7	Post Abs
3–8	35	4	1.37	250	585	0.66[2]	0.80	503	460	44.07	10.17	23.1	4¾ hrs.
3–10	36	6	1.38	237	608	0.73	0.81	498	423	50.55	11.08	21.9	Post Abs.
3–10	37	6	1.39	252	616	0.65[2]	0.80	341	220	47.37	11.11	23.5	4 hrs.
3–13	38	9	1.39	233	575	0.73	0.77	338	228	54.16	11.19	20.7	Post Abs.
3–14	39	10	1.40	237	609	0.70	0.78	505	492	53.79	11.20	20.8	Post Abs.
3–14	40	10	1.39	238	600	0.73	0.78	289	283	54.01	11.16	20.7	5 hrs.
3–15	41	11	1.30	230	535	0.67[2]	0.78	409	306	52.42	10.40	19.8	Post Abs.
3–15[3]	42	11	1.43	271	539	0.71	0.76	408	355	40.49	9.17	22.7	4 hrs.
Average, excluding No. 42			1.38	237	590	0.71	0.79	438	357	50.98	10.93	21.5	

[1] Aciduria began
[2] Probably invalidated by an unusually high initial metabolism.
[3] Calculated at 0.707, the R.Q. of pure fat combustion.

there is an obvious decline in efficiency as the series progressed both before eating and after. From the fourth and sixth days of the diet (March 8 and 10) to the eleventh (March 15) there was a falling off in the post-absorptive group of 2.0% (21.8 − 19.8) absolute or 9.1% relative. Figuring the decrease for the experiments 4 to 5 hours after breakfast the loss in efficiency from the 4th and 6th days to the 10th and 11th, is 1.6% absolute or 6.1% relative. Without the last experiment the loss to the 10th day is 11.1%. Omitting this experiment the decline in both series is seen to be quite gradual and consistent.

In two other respects this high fat series is of particular interest in relation to the loss of efficiency. The non-protein R.Q. during work begins at the level of about 0.81 (average of first four experiments) and ends at 0.77+ (average of second four). Also the nitrogen excretion falls from pre-work to the work and recovery periods, much more in the first four (111 milligrams) than in the second four (56 milligrams). If the higher efficiency at the beginning is related to greater combustion of carbohydrate, and the greater sparing of protein is due to the same factor[1] are we not justified in saying that the greater efficiency is related to the diminished metabolism of protein?

Following the method of Frentzel and Reach (p. 121) for obtaining the difference in efficiency between pure fat and pure carbohydrate combustion, we find from Table II that an average R.Q. of 0.93 during work at the rate of 1.4 Cal. per minute is accompanied by an average net efficiency of 22.7%, while from Table III an average R.Q. of 0.79 during work at the same rate is accompanied by an average net efficiency of 21.5%. The difference in efficiency for a difference in R.Q. of 0.14 (0.93—0.79) is 1.2%. What would it be for a difference in R.Q. of 0.293 (1.00—0.707)?

$$1.2\% \div 14 = .0857\% \text{ for a unit change in R.Q.}$$
$$22.7\% + (.0857 \times (100 - 93)) = 23.30\%$$
$$21.6\% - (.0857 \times (79 - 70.7)) = \underline{20.80\%}$$
$$2.5\%, \text{ difference between}$$

pure fat and pure carbohydrate

Now 2.5 is 12.02% of 20.80 and 10.73% of 23.3. Therefore pure carbohydrate combustion is approximately 12% more efficient than pure fat or, pure fat is approximately 11% less efficient than pure carbohydrate according to the basis of comparison. The figures agree very well with those of Frentzel and Reach (12.8%) and of Krogh and Lindhard (11.25%)

[1] Gaebler and Murlin, (Journ. Biol. Chem., 1925, LXVI, 731) have shown that the sparing of protein is greater when carbohydrate actually burns than when it is merely present in circulation and not burned.

Second High Carbohydrate Diet

It was deemed best to run another series on both the carbohydrate and fat diets in order to check the first results. The high carbohydrate diet accordingly was resumed on the 16th of March and six experiments were run in the next four days. They show (Table IV) the same variation as the first series on high carbohydrate. The standard deviation is 0.96 and the coefficient of variability 4.3%. However, the subject was still feeling a little heavy from the effects of the fat diet, with occasional dizziness during the work period, and was not able to do the work with ease as he had on the first carbohydrate diet. It was decided, therefore, to allow the subject two or three days in which he might eat anything he liked in an attempt to get back to normal. The carbohydrate diet was then resumed on the 25th of March but the work was reduced by diminishing the speed from one complete revolution every second to a revolution every one and a quarter seconds, thus cutting down the work from 1.4 Cals. per minute to 1.1 Cals per minute. The total time was kept the same, namely eight minutes and the recovery period also about the same. As a result there was a surprising increase in efficiency, an average of 23.4% as contrasted with 22% before the change and with an almost constant value for the four experiments. The work having been changed, it is only the last four experiments which are strictly comparable with the next series on the fat diet.

The effect of the previous fat diet is seen in the initial non-protein R.Q. for the first two experiments. Indeed the average for the first six experiments in the first four days was 0.89 while in the last four experiments after the work had been changed the initial R.Q. was 0.94. The average is the same in both series during the work and recovery period, namely 0.96. The decrease in nitrogen excretion in the work and recovery period as compared with the pre-work period in this series is not so great as in the previous carbohydrate series, probably because of the hold-over effect of the fat diet. That the amount of work has no particular relation to this decrease in protein metabolism is witnessed by the fact that in the first six experiments where the work averaged 1.38 Cals. per minute the decrease is 74 milligrams while in the last four experiments, where the work averaged 1.1 Cals per minute, the decrease is 77 milligrams per hour. Where the initial R.Q. is already high, as in the last four experiments, work of moderate intensity does not call for an increased mobilization of glycogen and therefore the change in sparing of protein is negligible. Probably the decrease in nitrogen excretion in the entire series is due merely to the normal decline taking place from hour to hour

TABLE IV

METABOLISM AND NET EFFICIENCY—CARBOHYDRATE DIET, SECOND SERIES

Date 1927	Ex. No.	Day of Diet	Work Cal. Per Min.	Total O_2 cc. per min.		Non-Prot. R.Q.		N. Excr. Mgm. per Hr.		Excess Cal.	Total Work Cal.	Net Eff. %	Hrs. aft. breakfast or post absorptive
				Pre Work	Work & Recov.	Pre Work	Work & Recov.	Pre Work	Work & Recov.				
3–17	43	2	1.39	235	573	0.73	0.89	448	280	51.13	11.19	21.9	Post Abs.
3–17	44	2	1.39	251	552	0.82	0.93	489	351	47.32	11.15	23.6	4 hrs.
3–18	45	3	1.40	219	505	0.92	0.95	464	584	39.95	8.79	22.0	Post Abs.
3–18	46	3	1.39	229	546	1.00	0.98	386	358	49.24	11.13	22.6	4½ hrs.
3–19	47	4	1.36	224	541	0.77	0.93	492	278	50.12	10.91	21.8	Post Abs.
3–21	48	6	1.38	189	547	1.14	1.06	441	428	54.44	11.10	20.4	Post Abs.
Average			1.38	224	544	0.89	0.96	453	379	48.70	10.71	22.0	
3–25	51	1	1.10	215	445	1.05	0.97	374	427	37.47	8.80	23.5	Post Abs.
3–25	52	1	1.09	231	470	0.95	0.97	474	278	37.58	8.79	23.4	4 hrs.
3–26	53	2	1.10	233	457	0.78	0.92	506	402	37.76	8.83	23.4	Post Abs.
3–27	54	3	1.09	205	431	0.95	0.97	359	300	37.61	8.79	23.4	Post Abs.
Average			1.09	221	450	0.93	0.96	428	351	37.60	8.80	23.4	

since the ingestion of the last food. Indeed, seven of the ten experiments were post-absorptive and in two of these, one in the first six experiments, and one in the last four, the excretion of nitrogen during work and recovery is actually higher than in the pre-work period. We are disposed to look upon this result as due to imperfect collection. In all of the other experiments the nitrogen excretion in the work and recovery period is distinctly lower than in the pre-work period.

Second High Fat Diet

The second high fat diet was started on March 29 and was continued for seven days (Table V). The average net efficiency in this series is 22.6%. Again we observe a noticeable decline in the efficiency from the beginning to the end of the series. For example, from the third day of the diet (March 31) to the last day (Apr. 4) there is a decline in the efficiency of the experiments done from four to five hours after breakfast from 23.2 to 21.5%, a loss of 7.3% relative, as compared with a loss of 11.1% from the fourth and sixth to the tenth day of the first high fat series. The post-absorptive experiments in this last series did not give so clear a result as in the first fat series, probably because the series was not prolonged far enough and possibly also because the amount of work was significantly less.

This final series is remarkably concordant as regards the non-protein R.Q. during the work and recovery period (Table V). Even here, however, we may note a decline from the first experiment where the quotient was 0.81 to the last where it was 0.75. We are doubtless justified, therefore, in referring a part of the decline in efficiency to increased dependence on fat during work.

The average decrease in nitrogen excretion during the work and recovery period as compared with the pre-work period in this series is only 44 milligrams, a result which again is plainly associated with diminished combustion of carbohydrate during work. Comparing the first four experiments in this series having an average net efficiency of 23.0% with the last four having an average of 22.2%, the average diminished nitrogen excretion in the first four is 60 milligrams and in the last four 31 milligrams. The comparison is somewhat vitiated unfortunately by the extraordinarily high excretion in the work and recovery period of the 63rd experiment (April 2).

Fig. 1, represents graphically the loss of efficiency with the progress of the experiments in the two high fat series. Four of the lines exhibit a strikingly similar trend—so much so that it would seem to be justifiable to regard them as representing proportional changes in the post-absorptive

TABLE V

METABOLISM AND NET EFFICIENCY—FAT DIET, SECOND SERIES

Date 1927	Exp. No.	Day of Diet	Work Cal. Per Min.	Total O_2 cc. per min.		Non-Prot. R.Q.		N. Excr. Mgm. per Hr.		Excess Cal.	Total Work Cal.	Net Eff. %	Hrs. aft. breakfast or post absorptive
				Pre Work	Work & Recov.	Pre Work	Work & Recov.	Pre Work	Work & Recov.				
3–29	Started the diet.												
3–30	58	2	1.10	228	465	0.79	0.81	426	379	37.84	8.79	23.2	Post Abs.
3–31[1]	59	3	1.08	231	468	0.74	0.77	465	431	37.50	8.71	23.2	Post Abs.
3–31	60	3	1.10	241	480	0.73	0.77	468	309	38.06	8.82	23.2	5 hrs.
4–1	61	4	1.09	230	478	0.72	0.77	494	503	39.31	8.75	22.3	Post Abs.
4–1	62	4	1.09	242	497	0.74	0.77	297	281	37.93	8.74	23.0	Post Abs.
4–2	63	5	1.09	238	480	0.71	0.77	484	608	39.54	8.74	22.1	Post Abs.
4–3	64	6	1.10	244	491	0.70	0.76	379	330	39.21	8.78	22.4	Post Abs.
4–4	66	7	1.12	247	511	0.70	0.75	489	305	41.79	8.98	21.5	4 hrs.
Average			1.10	238	484	0.73	0.77	438	393	38.89	8.79	22.6	

[1]Aciduria began.

and after-breakfast groups of the two series respectively. However, the proportion is not quite exact. Thus in three days (2nd to 5th) of the post-absorptive group of the 2nd series the decline expressed as a percentage of the initial efficiency is 4.7. In five days (6th to 11th) of the same group, first series, a proportional decline would be 7.8%. The actual fall is 9.6%. Two causes contribute to the difference—heavier work and more prolonged subsistence on the high fat.

A comparison of the two groups of experiments made 4 to 5 hours after breakfast shows a different result. In four days of the 2nd series (3rd to 7th) the decline is 7.3%. A proportional decline for 6 days would

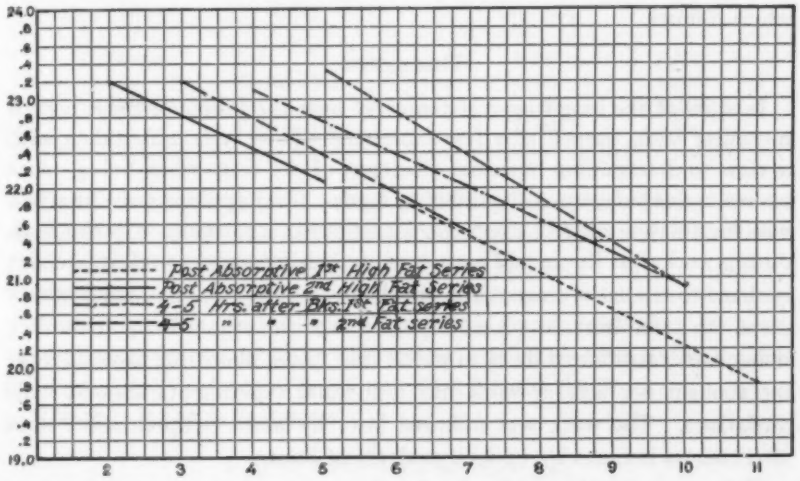

FIG. 1. Graph showing the progressive decline in efficiency on high fat diets. Abscissae are days of the diet; ordinates percentage net efficiency.

be 10.9%. The actual result for the apparently parallel curve in the first series was (4th to 10th day) 10.4%. However, as we have seen above (page 112) a truer comparison probably would be to take the average efficiency of the 4th and 6th days as the starting point (since the non-protein work R.Q. on the 6th day is still 0.80) and the efficiency of the 10th day 20.7% as the end-point (since the corresponding experiment of the 11th day was stopped prematurely). This gives the result shown in the steepest curve of the chart. If it is fair to take the average of the 4th and 6th days, we must count from the fifth. Consequently the fall in five days (11.1%) is at a much higher rate than in the 2nd series where the rate

of work was 21% less and the period of sustenance on fat four days shorter. Adopting this latter figure as the truer result it turns out that the loss of efficiency is more rapid in both groups as the high fat diet is prolonged. It was unfortunate that the subject could not stand the higher rate of work throughout.

The graphs in Fig. 1 emphasize another important result of this study; namely, that there is no loss of efficiency definitely discernible until the fourth day of the high fat diet. The cause of this might appear to lie in the fact that the diet though high in fat, still contained sufficient carbohydrate to maintain, with the protein, the glycogen supply of the muscles, but further reference to this possibility will be made in the discussion which follows.

Discussion

In spite of the long-waged controversy over the source of the energy of muscular work, surprisingly few experiments have been undertaken with a view to determining muscular efficiency of the body when living, more or less exclusively, upon the different foods. The hypothesis of Chauveau was formulated entirely upon considerations of the changes in the R.Q. during work; but Zuntz and his school in their attempts to refute the idea resorted to studies of the economy of the body on fat and on carbohydrate diets. In 1894 Zuntz and Loeb reported experiments made upon the dog doing work on different diets, in which they found about the same average metabolism per unit of work but individual experiments showed wide variations. The work was followed later by more comprehensive data obtained on humans by various students of Zuntz.

Heineman (1901) working in the Zuntz laboratories reported a long series of experiments extending over a period of 4 months, on a man working on an arm ergostat and subjected to different diets. He gives the following summary which indicates a greater economy upon the fat diet of about 12%.

Diet	R.Q.	Cal./KgM. work.
Fat	0.72	9.27
Carbohydrate	0.90	10.37
Protein predominating	0.80	10.64
Fat and carbohydrate	0.78	10.35
Fat and carbohydrate	0.81	10.46

Zuntz (1901) in his discussion of this work suggests that this difference is probably due to training since the experiments on the fat diets were done after those on the carbohydrate diets. One has difficulty in making this out from the tables of Heineman, however.

In the same volume of Pflüger's Archiv is published the work of Frentzel and Reach (1901) also of the Zuntz school. They report 176 experiments made upon themselves on carbohydrate and fat diets while doing work on a tread mill inclined at an angle of about 13 degrees. The following is a summary of their results.

Subject	Diet	Work R.Q.	Cal./ KgM.
Frentzel	Fat	0.773	2.066
	Carbohydrate	0.889	1.980
Reach	Fat	0.781	2.119
	Carbohydrate	0.900	2.086

Frentzel showed slightly better utilization of carbohydrates for the support of work but the difference found in the experiments on Reach were within the range of deviations between series.

Since the 29 or 30% loss of energy on a fat combustion postulated by Chauveau was for pure fat and pure carbohydrate combustion, the authors calculate what should be the energy loss when the quotients were 0.707, and 1.00 instead of (for Frentzel) 0.773 and 0.889.

The calculation is as follows:

R.Q. of 0.773 required 2.066 Cal.
R.Q. of 0.889 required 1.980 Cal.

Difference 116 0.086 Cal.

$\frac{0.086}{116}$ equals .0007414 Cal. difference for a unit change in R.Q. On this basis for a pure fat combustion with R.Q. .707 we have $2.066+(.0007414\times(773-707))=2.114$. For carbohydrate with R.Q. of 1.00 we have $1.980-(.0007414\times(1000-889))=1.898$. $2.114-1.898$ gives a difference of 0.216 or 12.8% between pure fat and pure carbohydrate combustion.

Since this is so much below the value of 30% of Chauveau they feel justified in concluding, "dass Fett und Kohlenhydrate bei ihrer Oxydation im Thierkörper in nahezu gleich ökonomischer Weise entsprechend ihren calorischen Werthen kinetische Energie zu liefern im Stande sind."

In the experiments of Benedict and Cathcart (1914) upon the bicycle rider M.A.M., a few were made on carbohydrate-rich and carbohydrate-poor diets. The experiments are not at all conclusive, as the authors state. The latter diet was never free of carbohydrate, a quotient of 0.84 was obtained in one case and never was it below 0.77. There is wide variation also but the averages are given below.

As the authors state, the experiments "showed not the slightest indication of an increase in the energy output per unit of work" for one foodstuff as compared with another.

Diet	Resting R.Q.	Work (Cal.)	Cal. per Unit of Work
Carbohydrate rich	0.85	2.10	5.37
Carbohydrate poor	0.81	2.07	5.31
Carbohydrate rich	0.89	1.54	6.66
Carbohydrate poor	0.77	1.57	6.68

Krogh and Lindhard had chosen to use the R.Q. during the activity period as an index of the nutritive state and by arranging these according to the quotients in Benedict and Cathcart's work find, "that they are too irregular to admit of any conclusions, but that they are perfectly compatible with our results; that there is a definite wastage of energy derived from fat."

With a dog running on a tread mill, Anderson and Lusk (1917) have shown that after an eighteen hour fast it required an extra expenditure of 0.580 kilogram meters to move one kilo of the dog's weight through one meter. After the ingestion of 100 grams of glucose the expenditure was reduced to 0.550. This represents a 5% difference, but Krogh and Lindhard calculate that if the metabolism per unit of work is assumed to be a straight line function of the quotient, the waste of energy for fat works out as 8%.

The work of Krogh and Lindhard (1920) consisted of four series of experiments of about 220 determinations made upon six subjects. The subjects rode the bicycle ergometer placed within a Jaquet respiration chamber. The respiratory exhange was measured by analysis of the air withdrawn from the chamber. The subjects ate a low protein and fat diet or low protein-carbohydrate diet for two days previous to a determination and then, in most instances, continued the diet and worked on the ergometer for two days. They have, in two out of the six subjects, obtained very uniform results, but in all, the net expenditure of energy

per Cal. of work varied from 5.5 to 4.0 Cal. or expressed as net efficiency, from 18.2% to 25.0%. It varied with the subject and increased with training so that a correction for this factor was made. In the third and fourth series in which the results are most consistent, the wastage of energy from fat has been calculated to be 9.9%. If all the results are averaged it comes out 11.25%. In making up the averages account was taken of the number of determinations and the dispersion of these determinations.

The following year, Orr and Kinlock (1921) published results upon the influence of diet on energy expenditure of work. They determined the expenditure during rest and during walking (constant amount of work) by analyses of expired air collected in a Douglas bag. The same determinations were repeated 90 minutes after a test meal consisting of, (1) 50 grams of oatmeal plus 100 grams plasmon, (2) 80 grams of cane sugar, (3) 35 grams of margarin, containing 540, 540, and 480 calories respectively. All the experiments were made upon one subject and three experiments made upon each of the diets. A summary follows.

Meal	Net Expenditure of Work Cal. / Min.		Diff.
	Post Absorptive	After Meal	
Protein	3.86	3.96	+0.10
Carbohydrate	3.71	3.65	−0.06
Fat	3.77	3.77	0.00

The authors conclude that with protein there was more than summation of the extra metabolism due to food and that due to work; that there was an increased rate in the additional metabolism due to work thereby causing this greater heat production per unit of work. With carbohydrate as fuel the muscles worked more economically or, in other words, part of the increased metabolism due to food was available for work production. With fats there was simple summation.

The variations within each set of values obtained in this report of Orr and Kinlock are usually much greater than any difference which the averages show, so that little weight can be placed on these experiments.

The paper of Cathcart and Burnett (1926) contains only a few experiments upon fat diets, the authors being more interested in diets to which varying amounts of protein were added. However, two series of experiments on a fat diet (360 grams of olive oil a day for three days) showed the oxygen consumption per kilogram-meter of work done to be 2.70 and 2.36 respectively, whereas it was 2.64 cc for mixed diets. They also

calculated the absolute net oxygen utilization in cc. per kilogram-meter (namely load less no load values) and found it to be 0.36 in both cases, on mixed diet and on fat diet. It was slightly lower, .031, on the carbohydrate however.

Recently Reynolds, Sevringhaus and Stark (1927) have reported experiments made on themselves, planned to duplicate, so far as possible, the experiments of Krogh and Lindhard. The work was done on a bicycle ergometer described by Sevringhaus and determinations were made only of oxygen by the Benedict-Roth portable machine and the graphic method. Efficiencies were determined on a normal mixed diet, after a carbohydrate meal of glucose and after six meals on a high fat diet. They were obliged to assume an R.Q. since they had no value for the carbon dioxide. They used 0.84 rather than 0.82 which is usually assumed with that type of apparatus, because the subjects were not in the post-absorptive state. The determinations were made in the afternoon some time after lunch. They have calculated all their efficiencies (1) using a quotient of 0.84 and (2) using a quotient of 1.00 for the experiments following the glucose ingestion and 0.76 for the experiments following the fat. The authors conclude that there is little or no difference in the efficiency with changing diets.

There are several criticisms which might be made of this work. First, any experiments made upon muscular work without a determination of R.Q. must necessarily entail considerable error especially when diets of carbohydrate and fat are to be compared. In the second place, determinations were made on only one day on the carbohydrate and one day on the fat. Within four or six determinations made on these days there are such wide variations that the average value may not be the true mean value.

Nevertheless, the results obtained in the early days of our experiments substantiate very well the results of Sevringhaus, namely that there is little if any decrease in efficiency *up to the third day of the fat diet.* What the reason is for this difference found by Krogh and Lindhard on the one hand, and by Sevringhaus and ourselves on the other hand, is a question. In the experiments of Sevringhaus and in our own, the work was done with short periods. This may be one factor which could enter in to explain the difference. Another may be the diet. Neither Krogh and Lindhard nor Sevringhaus give the caloric values of their diets nor the ketogenic anti-ketogenic ratios. In the latter's experiments it seems that the ratio must have been high since ketosis developed after the third meal, but the former authors give no data concerning it. Our ketogenic-anti-ketogenic ratio as calculated by the Woodyatt (1921) formula was about 1.7,

a value only slightly above the border line of 1.5. If that of Krogh and Lindhard were considerably higher, and, if the lowered efficiency is in some way connected with a disturbed metabolism and its abnormal products, the delayed fall in efficiency in our work might be explained on this basis.

Possible Causes for Lower Efficiency on High Fat Diet

We shall consider three possible causes for the lower efficiency of high fat diets in supporting muscular work; (1) the conversion of fat to carbohydrate; (2) the acidosis; and (3) the lessened sparing of protein.

Regarding the relation of muscular efficiency to the conversion of fat to carbohydrate, it should be stated, in the first place, that there is no satisfactory direct proof that fat is so transformed at any time. Many investigators have been misled by the general chemical postulate that all reactions are reversible. In a system such as is maintained in the mammalian body there are many instances of reactions which go normally only in one direction and the conversion of carbohydrate to fat seems to be one of these. We have no intention here of going into the evidence as to whether fat is or is not at any time converted to carbohydrate. Since however, we are not even certain that the process does take place, we can only speculate as to how the process might go—what the path would be. Krogh and Lindhard did well in calling attention to the fallacy of making a paper and pen calculation of energy changes resulting from this transformation and cite as an illustration the possibility of changing one molecule of fat (tripalmitin) into twelve molecules of sugar by the addition of twenty-one molecules of CO_2 and twenty-three molecules of H_2O, which would result in a gain in energy of about 18% rather than a loss of 30% which the calculation of Chauveau called for. Thus, if the mammalian muscle can use only carbohydrate, and was under the necessity of transforming fat into carbohydrate in case the food did not supply a sufficient amount, there is the possibility that energy might be gained rather than lost and the body be even more efficient on a fat diet. Meyerhof and Himwich (1924) have commented upon this calculation and regard it as most unlikely that CO_2 is directly added to produce carbohydrate. They would rather believe that the carbon of sugar has all come from the carbon of fat. On the other hand, if carbohydrate to fat were demonstrably a reversibile reaction, we might expect Krogh and Lindhard's calculation to be more nearly correct since Bleibtreu (1901) has shown that it is the CO_2 output which is increased rather than the O_2 intake decreased when carbohydrate is changed to fat. Bleibtreu gives the following equation:

4 $C_6H_{12}O_6+O_2=C_{16}H_{22}O_2+8\ CO_2+8H_2O$. Since this reaction is exothermic (4.7% of the heat being liberated), if the process were reversed heat would be spared and CO_2 might be added.

Secondly, considerable evidence was presented in the former paper (Marsh, 1928) showing that muscles do oxidize fat even for short periods of work and even when carbohydrate is present in abundance, at least in some individuals. But if we assume for the moment that the muscle can use only carbohydrate, the question arises whether the amount of glycogen present can account for the persistently high efficiency on the first few days of the high fat diet. (See Table V.) Might there be after three or four days still enough available glycogen to furnish energy for the work and might its gradual exhaustion account for the progressively lower efficiency? The initial non-protein quotient starts (Table V) the second day of the diet at 0.79 and then falls rather abruptly the third day to 0.74 and 0.73. From this point it falls more gradually to 0.71 or 0.70 at the end of the series where the efficiency is 1.7% lower than at the beginning. Since the non-protein quotient of 0.79 represents the combustion of enough carbohydrate to yield 30% of the non-protein Calories, it would seem that the decrease in efficiency were running parallel with the decrease in glycogen stores. On this supposition we should expect that the R.Q. of the excess metabolism would be as high as on the carbohydrate diet and that it too would fall gradually. But this is not the case. In the first high fat series the excess R.Q. does run a little higher at the beginning than at the end (see Table 9 of earlier paper) but 0.86 is the highest, while in the second series there is a variation of only four points in the entire series and it is as low at the beginning (0.79) as at the end. (See Table 11 of earlier paper.)

As a further check on the question of glycogen storage the subject on March 31 between experiments 59 and 60, took a hard run outdoors for half an hour and thereby burned up a considerable amount of any available glycogen. He rested for two hours and in the following experiments showed exactly the same quotients and exactly the same efficiency as before the run. This makes it appear that stored glycogen was not playing any material part in keeping up the efficiency.

If the hypothesis of Chauveau, as stated above, were the correct one for explaining the lower efficiency, then for the first few days of the fat diet while efficiency is still high one would be obliged to assume that carbohydrate is furnishing most of the energy and that there is little or no conversion from fat taking place. But a definite aciduria was present on the third day of the diet while the efficiency was still high. There was an

initial R.Q. of 0.74 and 0.73 (see experiments 59 and 60, Table V), the R.Q. for the work and recovery period was 0.77 in both these experiments and the R.Q. of the excess metabolism was 0.79 in both. These signs certainly indicate fat combustion or carbohydrate combustion accompanied by transformation of fat to carbohydrate, if that foodstuff only can be oxidized. On the last-named supposition the transformation of fat into carbohydrate, however, would be taking place without a change in energy such as could be detected in the efficiency. From this it appears that we cannot account for the lower efficiency on Chauveau's theory.

We may next consider briefly the possibility that the acidosis, or the subjective symptoms of lassitude and fatigue, are responsible for the altered efficiency. There is no direct evidence that either of these would exert any influence. The fact that one has no desire to work does not of necessity mean that when one does work more heat would be produced than when one does the work with ease. Granted that it takes more will power; yet will power must be classed as a mental activity and the evidence for an increase in the total metabolism caused by mental work is as yet not conclusive. Johanssen (1901) reports that a subjective sense of strain or fatigue has no influence upon the metabolism.

That acidosis, per se, could make any difference seems unlikely if by acidosis we mean simply an increase in acid production, compensated or not. In the case of acidosis on fat diets the production of ketone bodies is largely responsible and in all probability in the amounts produced in these experiments was entirely compensated for by increased production of ammonia and probably also by respiratory change. The percentage of CO_2 in the alveolar air on the high fat diets was found to be significantly lower at the end of work and recovery than on the high carbohydrate diets (see Table 12 of previous paper). Moreover the alveolar CO_2 tension fell rapidly following work in one of the high fat series in sharp contrast with its behavior on the high carbohydrate diet which would indicate more rapid elimination of CO_2 than was normal. Indeed, as stated in the former paper the rise in R.Q. during work and recovery on the high fat diet cannot be accepted as evidence of increasing carbohydrate combustion because of the acidosis.

However acidosis means something more than slight changes in the production of acid bodies and their neutralization or elimination in the urine. The symptoms of langour, early fatigue, and unwillingness to undertake any activity, which were marked in both the high fat series reported here, indicate a toxic action of acid products only partially oxidized or only partially neutralized by the body's alkali. Peterman (1924)

in his discussion of this toxic action says that of the three substances acetone, diacetic acid and beta hydroxybutyric acid, only the second, diacetic acid, has any pronounced toxic action but that its effects are not unlike those of anesthetics. The similar solubilities and other physical and chemical properties amongst anesthetics indicate similarity of physiological action. Diacetic acid, like most anesthetics, is soluble in water and in fat. Most anesthetics possess a chemical structure indicating that they undergo a certain degree of dissociation into bivalent carbons or methlene-like products. Peterman believes that diacetic acid also in all probability undergoes this methylene dissociation. Hence the characteristic symptoms of acidosis may be due to real anesthetic effects of diacetic acid. Since its value as an anesthetic is supposed to lie somewhere between that of alcohol and that of chloroform, and alcohol is known to lower efficiency (Miles, 1924), possibly the diacetic acid, even in very small amounts might exert a like effect. Much more evidence would be required to place any such hypothesis on safe ground.

The lessened sparing of protein may detain us a little longer. Is there really a greater sparing of protein when work is done on high carbohydrate? The clearest difference in the reduction of protein metabolism is presented by the post absorptive experiments of the first high carbohydrate and first high fat series (Tables II and III). There are five of these in each series. Taking the figures from the columns headed "Total Work," "Excess Cal." and the difference between "Pre Work" and "Work and Recovery" under "N Excretion" we get Table VI. The average difference in extra metabolism is 3.22 Cal. The sparing of protein occasioned by the combustion of nearly pure carbohydrate as compared with nearly pure fat is 96 milligrams. For convenience this may be called 100 milligrams. Protein has participated in the metabolism, during work as compared with pre work, to the extent of 100 mgm. *per hour* more on high fat than on high carbohydrate. The duration of the experiment however is only 8 minutes of work and from 17 to 28 minutes of recovery. The extra metabolism is calculated always by subtracting from the total metabolism for work and recovery the resting metabolism for an equal length of time. Hence the higher protein metabolism during actual work and recovery on high fat is never more than 41 to 60 milligrams. However, at the rate of 26.51 Cal. for each gram of nitrogen excreted this comes to 1.09 and 1.59 Cal. for the two extremes, and would apparently account for from 34 to 49 (average 42) per cent of the difference in extra metabolism found from Table VI. By a similar calculation we can account for 52% of the average difference in extra metabolism between the post-absorptive

experiments of Table IV (High carbohydrate) and Table V (High fat) by the change in lowering effect on the protein metabolism.

There are two ways of looking upon this fraction of the protein metabolism apparently spared by the high carbohydrate as compared with the high fat diet. If it is a fraction of the total metabolism which otherwise would fall upon non-nitrogenous food (e.g. carbohydrate, as if this were necessary in effecting the oxidation of fat) there is really no ad-

TABLE VI

COMPARISON OF POST ABSORPTIVE EXPERIMENTS FROM TABLE III, HIGH FAT, FIRST SERIES

Exp. No.	Total Amt. of Work Cal.	Extra Metab. Occasioned by Work. Cal.	Difference in N Excretion Between Pre work and Work and Recovery Mgm.
34	11.18	51.44	205
36	11.08	50.55	75
38	11.19	54.16	110
39	11.20	53.79	13
41	10.40	52.42	103
Average	11.01	52.47	101
	FROM TABLE II, HIGH CARBOHYDRATE, FIRST SERIES		
25	11.20	45.88	134
27	11.22	49.38	111
29	11.16	51.71	233
31	11.15	52.03	209
32	11.20	47.26	300
Average	11.18	49.25	197

vantage in the sparing effect. This will be clear from the following consideration. In the calculation of the total metabolism the oxygen allotted to protein (8.45 gm. per gram of nitrogen in the urine (Lusk, 1917)) is deducted from the total, leaving less oxygen to be used in the computation of the non-protein metabolism. Hence if in one series 100 milligrams of nitrogen less than in another came from protein as a part of the extra metabolism due to work, it would correspond to 0.592 (0.845×0.7) L. of oxygen less to be deducted from the total—in other words leaving this much more to be added to the non-protein metabolism. At a non-protein R.Q. of 0.93 (the average of the high carbohydrate work periods) the 0.592 L. of oxygen would have a heat value (Lusk, 1924) of ($0.592 \times 4.961 =$) 2.937 Cal. The net effect therefore would be the sparing

(as calculated) of 2.651 Cal. per hour from protein and the production (as calculated) of 2.937 Cal. from non-nitrogenous sources (chiefly carbohydrate). So far from accounting for the greater efficiency of high carbohydrate therefore we should have a calculated heat production (2.937 − 2.651 =) 0.286 Cal. per hour higher on this diet than if the protein had burned. Calculated as a sparing on fat (non-protein R.Q. of 0.79) and a waste heat production from protein the difference would be (2.834 − 2.651 =) 0.183 Cal. per hour lower than if the protein had not burned. Fat combustion in this view of the matter is more economical than carbohydrate.

If, however, the diminished protein metabolism on high carbohydrate represents a fraction which is additive—something which takes place merely from the presence of more protein split products in circulation, when fat is the main source of energy, the total oxygen absorption would be greater (on fat) and the efficiency would be less as calculated on page 128. The problem is complicated by the difficulty of arranging adequate control experiments. To determine whether the same differences in nitrogen excretion would obtain whether or not work was done would require a control series of observations made in the post-absorptive state, but with the subject resting on the bicycle ergometer instead of working, and carried out at corresponding hours of the day with the work experiments. To be adequate the control series should be equal in length to, and alternate day by day with, the work series and this arrangement would prolong subsistence on the experimental diets quite too far, since the high fat diet in particular is so difficult to endure. To determine whether the protein fraction of the extra metabolism were additive or substitutive would require direct as well as indirect calorimetry for both control and work experiments and the prolongation of the latter to the duration of at least one hour, in order that differences due to the change in sparing effect should be measurable as heat.

We reach a somewhat clearer view from another way of looking at the problem. When the distribution of calories to the three organic food stuffs (Table VII) is calculated for the work and recovery period and compared with the distribution found in the pre-work period for each of the post-absorptive experiments,[2] it is found, as shown in this table, that the average percentage change in all the foodstuffs is higher for the high carbohydrate experiments, than for the high fat.

[2] Experiment 34 which appears in Table VI is omitted from Table VII because the subject had not yet sufficiently recovered from the effects of the previous high carbohydrate diet, as shown by the non-protein R.Q.

TABLE VII

DISTRIBUTION OF CALORIES IN RESTING AND WORK AND RECOVERY PERIODS POST ABSORPTIVE EXPERIMENTS, HIGH CARBOHYDRATE, FIRST SERIES

Exp. No. and Period	Non-Prot. R.Q.	Carbohydrate		Fat		Protein		Total Cal.
		Cal.	% of Total	Cal.	% of Total	Cal	% of Total	
25 Pre Work	.77	5.1	17.7	17.2	60.1	4.32	22.2	28.66
Work & Recov.	.96	60.7	81.4	8.9	12.0	4.87	6.6	74.54
Change		+55.6	+63.7	− 8.3	−48.1	+0.55	−15.6	+45.88
27 Pre Work	.73	1.8	6.5	19.1	70.8	6.15	22.7	27.05
Work & Recov.	.91	50.6	66.2	20.9	27.3	4.95	6.5	76.43
Change		+48.8	+59.7	+ 1.8	−43.5	−1.20	−16.2	+49.38
29 Pre Work	.87	10.0	41.2	7.40	30.4	6.89	28.4	24.30
Work & Recov.	.97	64.8	85.2	6.88	9.0	4.32	5.8	76.01
Change		+54.8	+44.0	− 0.52	−21.4	−2.57	−22.6	+51.71
31 Pre Work	.86	11.26	43.7	9.55	37.1	4.94	19.2	25.75
Work & Recov.	.89	48.3	62.1	26.9	34.6	2.54	3.3	77.78
Change		+37.04	+18.4	+15.35	− 2.5	−2.40	−15.9	+52.03
32 Pre Work	.92	15.6	55.1	5.46	19.2	7.29	25.7	28.37
Work & Recov.	.91	50.9	67.3	21.0	27.8	3.71	4.9	75.63
Change		+35.3	+12.2	+15.54	+ 8.6	−3.58	−20.8	+47.26
Average Change %			+39.6		−21.4		−18.2	
36 Pre Work	.73	2.08	6.7	22.7	73.4	6.16	19.9	30.96
Work & Recov.	.81	28.2	34.5	48.1	59.0	5.23	6.5	81.51
Change		+26.12	+27.8	+25.4	−14.4	−0.83	−13.4	+50.55
38 Pre Work	.73	2.6	7.3	28.5	79.1	4.93	13.6	36.00
Work & Recov.	.77	19.8	21.9	67.0	74.3	3.32	3.8	90.16
Change		+17.2	+14.6	+38.5	− 4.8	−1.61	− 9.8	+54.16
39 Pre Work	.70	0.0	0.0	26.3	79.7	6.69	20.3	33.01
Work & Recov.	.78	21.1	24.3	59.2	68.2	6.52	7.5	86.80
Change		+21.1	+24.3	+32.9	−11.5	−0.17	−12.8	+53.79
41 Pre Work	.70	0.0	0.0	31.55	83.1	6.42	16.9	37.97
Work & Recov.	.78	22.5	24.9	63.1	69.8	4.8	5.3	90.39
Change		+22.5	+24.9	+31.55	−13.3	−1.62	−11.6	+52.42
Average Change %			+22.9		−11.0		−11.9	

If we may suppose that the initial effect of work on metabolism is to increase the oxidation of glycogen (actually burned as lactic acid) (Hill, 1924) and that an immediate consequence of this is a sparing effect of protein, the difference presented by the two groups may be characterized tersely as *double* the substitutive and sparing effect for high carbohydrate as for high fat. On the former, work has caused an increase in the oxidation of carbohydrate of, roughly, 40% (total calories is the basis) and a decrease in the oxidation of fat and protein of (again roughly) half this. On high fat the same amount of work caused an initial change toward carbohydrate combustion of 22.9% and a substitutive change on fat and the sparing on protein of just over 11%. The rise in percentage of carbohydrate calories due to work must obviously be compensated by an equal fall in total percentage of the protein and fat calories, but it is a singular fact that the distribution of this fall should be, in the average, so nearly equal to the protein and fat components. In computing the distribution of calories the fraction of the oxygen belonging to protein metabolism is deducted first from the total and the remainder apportioned according to the non-protein R.Q. to the other two foodstuffs. The protein calories are then calculated on the basis of the nitrogen excretion. There is therefore nothing inherently predisposing to such a result in the method of calculation. The resulting change in distribution of calories due to work has just been characterized as double the substitutive effect of carbohydrate in place of fat and double the sparing effect on protein when carbohydrate is the main source of energy as when fat is the main source. This relationship is probably accidental. A difference in the total quantity of work or in the rate of work probably would alter the ratio. But the substitution of so nearly the same percentage of carbohydrate calories for fat calories and for protein calories in the two groups of post-absorptive experiments would seem to indicate some general principle of substitution. The individual experiments, however, vary widely and those of the carbohydrate series are less concordant than those of the fat series. Hence it will require many more experiments to establish a general law of change in metabolism caused by work.

If we are correct in assuming that the primary consequence of increasing the carbohydrate calories is to spare protein calories, the effect upon fat calories follows of necessity. Can it be merely fortuitous that the average increase in percentage of carbohydrate calories should cause a sparing in both series of so nearly one-half the corresponding average percentage of protein calories?

That the change in protein metabolism bears no relation to the altered fat metabolism is readily discernible also from Table VII. In the high carbohydrate series the fat metabolism is increased in the absolute sense in three and decreased in two of the five experiments. The average change is an increase of 2.73 Cal. for the period. In the high fat series the fat metabolism increases greatly in the absolute sense in all experiments, the average increase being 32.1 Cal. There is no proportion between these figures and those representing the altered protein metabolism, whereas both relatively and absolutely the altered protein metabolism bears an equal relationship to the altered carbohydrate metabolism in the two series.

On the absolute basis Table VII shows that the protein metabolism in the pre-work period was at the same level (average) in both series of the post-absorptive experiments, 5.91 Cal. in the high carbohydrate series and 6.05 Cal. in the high fat. When work was done the protein calories decreased on the average 1.84 Cal. (for the same length of period) on high carbohydrate and 1.09 Cal. on high fat. In a previous paragraph where this change was described in connection with Table VI in terms of milligrams of nitrogen excreted, it was spoken of unequivocally as a greater "sparing effect" for the carbohydrate diet. As we have just seen, however, it proves to be a similar change in relation to change of carbohydrate combustion in the two series. Comparing the absolute change in protein metabolism of 1.84 Cal. in the high carbohydrate and 1.09 Cal. in the high fat series with the absolute change in carbohydrate calories in the corresponding experiments, we find additional reason for regarding them as similar effects. For the increase in total carbohydrate calories in the former is (average) 46. 3 Cal. and in the latter 21.75 Cal. The ratio of carbohydrate increase to protein decrease is 25 to 1, in the first, and 20 to 1 in the second series. An increased combustion of 25 carbohydrate calories on the high carbohydrate diet and 20 on the high fat has caused a sparing of one protein calory. The result is closely similar in the two, but again is slightly favorable to the high fat diet. If a relative sparing of protein were essential to better efficiency we should have slightly better efficiency on high fat than on high carbohydrate. However, it is the absolute change in heat production which affects the efficiency and if the diminished protein metabolism (on high carbohydrates) or the increase (on high fat) does not merely take the place of non-protein metabolism, the diminished total in the former and increased total in the latter case must be a factor in the efficiency results.

Clearly the "sparing" of protein is no greater in relation to carbohydrate metabolism when work is done on high carbohydrate than when done on

high fat, though it is greater in relation to total metabolism. Until direct calorimetric measurements are available it cannot be stated with certainty whether under the influence of work the altered protein metabolism should be regarded as substitutive (for non-protein metabolism) or additive. There remains the possibility that the lower efficiency on high fat diet is due to a higher metabolism caused by a greater concentration of both protein and fat in circulation and consequently greater specific dynamic action of these food stuffs when work is done on high fat than when done on high carbohydrate. It would require blood analyses for amino acid nitrogen and fat to make sure of this. The only direct experiments of this nature which we can cite are the rather cursory observations of Murlin and Riche (1916) on the fasting dog while working on the treadmill, where it was shown that work increased the blood fat.

Many investigators have reported a delayed increased excretion of nitrogen during work, but this applies always to work which has been either very severe or much more prolonged than the experiments we report. Cathcart and Burnett (1926) have been able to show that for an hour's work of 25,000 kgm. meters performed on an arm ergostat for six successive days, there was a definite rise in the nitrogen output during the work days, but little or no increase in the four post-work days except in a single series out of four. The amount of work employed by these authors was at least five or six times that exacted of our subject. In the present series there was no sign of a delayed excretion of nitrogen caused by the work. It is fairly certain that if such had been the effect, it would have been detected in urines collected in the afternoon alternatively on the days when work was done in the morning and the days without work. Moreover, the increase reported by Cathcart and Burnett was very slight. Cathcart puts forward the view that there is a definitely enhanced protein metabolism as a result of muscular work, but that catabolic activity is nearly balanced by an anabolic activity; and this view is supported by the experiment of Bornstein (1901) who has demonstrated an actual retention of nitrogen in the hypertrophy of muscle resulting from exercise. But the catabolism however large, if balanced by anabolic activity, can have no effect upon the total energy exchange and can therefore have no bearing upon the point in question.

Summary and Conclusions

1. Forty-seven experiments on the muscular efficiency of a single subject while riding the bicycle ergometer are reported.

2. Twelve of the experiments were made while the subject was subsisting on a normal *ad libitum* diet. The last seven of these served as a basal series with which to compare subsequent experiments while the subject was subsisting on high carbohydrate and high fat diets.

3. The high carbohydrate diet contained 2400 Cal. daily of which 80% came from carbohydrate and approximately 12 and 8% respectively from fat and protein. The high fat diet contained 2800 Cal. of which 80% came from fat and approximately 12 and 8% respectively from carbohydrate and protein.

4. Two series of experiments were run on each of these diets, nearly equally distributed between post-absorptive experiments and experiments taken 4 to 5 hours after a meal rich in carbohydrate or fat.

5. The work was of the same duration (eight minutes) followed by a recovery period, in the same metabolism period, of 17 to 25 minutes on each diet, but differed as regards rate in the first and second series on each diet.

6. Urine was collected in two periods, the first including the pre-work resting period and the second the work and recovery as well as a second post-work resting period. The calculation of efficiency included the protein metabolism in all experiments.

7. The extra metabolism due to work is found by subtracting from the total metabolism for the work and recovery period, the pre-work resting metabolism calculated each time to the same length of period.

8. The average net efficiency on the normal diet at the rate of 1.4 Cal. of work per minute was 22.1%; on high carbohydrate at the same rate, 22.7%; on high fat at the same rate 21.5%. At the lower rate of 1.1 Cal. of work per minute the average net efficiency on the same high carbohydrate diet was 23.4%, and on the high fat 22.6%.

9. On the high carbohydrate diet there was no progressive change in the efficiency, but on high fat the efficiency progressively declined from the 3rd or 4th day of the diet to the 7th in one series and still more to the 11th day in the other.

10. The average difference in efficiency between high carbohydrate and high fat first series, calculated to pure carbohydrate and pure fat combustion, was 11 or 12% (relative), agreeing with previous results found by Frentzel and Reach (1901) and by Krogh and Lindhard (1920).

11. A difference in the diminution of nitrogen excretion from pre-work to work and recovery periods was noted on the high carbohydrate as compared with the high fat diet. This difference is due to the greater

sparing effect of increased carbohydrate combustion. Relative to the increase in carbohydrate calories, the decreased protein metabolism was the same on both diets, but since it is total metabolism which affects efficiency, the effect of the greater sparing of protein (on high carbohydrate) depends upon whether the protein calories replace non-protein under the conditions of these experiments. Direct calorimetry would be required to settle this question.

12. The effect of the specific dynamic action of higher protein and fat metabolism would require blood analyses which were not attempted in these experiments.

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VITAMIN REQUIREMENTS OF NURSING YOUNG

III. A QUANTITATIVE BIOLOGICAL METHOD FOR THE STUDY OF VITAMN B[1] REQUIREMENTS OF NURSING YOUNG OF THE ALBINO RAT[2]

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IN RECENT communications (1) I have demonstrated that the large requirements of vitamin B for lactation are due to the inability of the lactating mother to transfer that vitamin quantitatively in the milk and that infant mortality during the nursing period can be considerably reduced by a distribution of vitamin B between mother and nursing young according to needs; also that rearing of the young can be markedly expedited provided the nurslings receive daily such amounts of vitamin B as meet their optimum requirements. Such facts became apparent after the development of a quantitative biological method for the study of vitamin B requirements for lactation (2). Since the Quantitative Biological Method for the Study of Vitamin B Requirements of Nursing Young presented in this communication is an elaboration of the method perfected for studies of lactation, in order to have a clear understanding of detailed modifications, the general technique of procedure already described elsewhere is again summarized here as follows: Mothers with their litters, reduced to six in number, are transferred from our stock colony diet No. 1 (3) to a vitamin B-deficient ration, consisting of casein (purified), 20%; agar-agar, 2; McCollum's salt mixture 185, (4) 4; butter

[1] The term "vitamin B" is used in this and the paper following to signify the combination of the labile-antineuritic and the stable factors associated with the vitamin B complex, both of which are necessary for growth. We are at present engaged in differentiating the requirements of nursing young with respect to these two dietary factors, and our findings along this line will be presented in subsequent communications.

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fat, 5; and dextrin, 69. Mothers and young are placed in individual compartments on raised screens. Galvanized boxes provided with shavings and excelsior serve as comfortable nests for mother and young. Distilled water is furnished daily with traces of iodine in the water once a week. Daily records are taken of food consumption and mothers and litters are weighed daily and regularly between 8 and 10 in the morning. On such a dietary regime the mothers rear their young for a period of seven to fourteen days, depending on the amount of storage, during which period the mothers lose from 20 to 30 per cent of their body weights in their attempts to prolong lactation. At this point the young then reach a maintenance curve showing depletion of the vitamin reserves of the mother. It was found that from that critical point on throughout the nursing period the lactating mother required daily 500 mg. of a yeast concentrate (2) when all the vitamin B had to be supplied to the nursing young through the mother's milk. On the other hand, a total of only 180 mg. of this yeast concentrate distributed between mother and young, the mother receiving a daily allowance of 20 to 30 mg. and the litters of 6 young 150 mg., or 25 mg. daily to each nursling, provided enough vitamin B for rearing and weaning of young and at the same time supplied enough vitamin for maternal welfare.

The purpose of this investigation was to determine the minimum daily vitamin B requirement of nursing young during various stages of the lactation period, but particularly during the later stages, and from such findings develop a vitamin B unit to serve as a quantitative index in evaluating the potency of vitamin B concentrates. A dehydrated baker's yeast, Federal brand, (5) was used for that purpose. The vitamin B concentrate was prepared according to a method of Seidell (6) (modified by us) by adsorption on fuller's earth and by releasing the vitamin with sodium hydroxide, neutralizing the alkali with calculated amounts of sulphuric acid. In this preparation we used 50 per cent alcohol as the extractive instead of an aqueous solution and different proportions of fuller's earth from those employed on previous trials. This yeast concentrate was designated as No. 18.

At first we conducted experiments to determine the minimum daily requirement of this concentrate for lactation when administered to the lactating mothers at the point of depletion of their vitamin B reserves, and it was found that six mothers failed on a 200 mg. daily dosage, six failed on a 300 mg. daily allowance, and four failed on a daily administration of 500 mg. We then attempted a distribution of the vitamin preparation between lactating mother and nursing young according to needs. This

TABLE I

LACTATION RECORD OF FEMALES 12 AND 13 (FIRST AND SECOND LITTERS) TRANSFERRED FROM STOCK COLONY NO. 1 TO VITAMIN B DEFICIENT RATION 1009[2]

DISTRIBUTION OF VITAMIN B BETWEEN LACTATING MOTHER AND NURSING YOUNG ACCORDING TO NEEDS

(YEAST CONCENTRATE NO. 18)[3]

Date (1927)	Female No. 12[1]	Litter	Food consumed during previous 24 hrs.	Female No. 13[1]	Litter	Food consumed during previous 24 hrs.
Nov.	gm.	gm.	gm.	gm.	gm.	gm.
17	280			230		
18	228	29		190	25	
19	216	32	2	185	28	0
20	206	34	8	187	22(5)[7]	6
21	200	42	10	186	25	7
22	198	50	8	182	28	5
23	190	55	5	182	32	6
24	188	57	11	180(1)	34	6
25	183	63	14	180	36	10
26	178	72	8	180	41	6
27	176	79	19	172	46	4
28	179	84	7	169(2)	50	10
29	168(3)	86	7	162	53	11
30	175	91	16	162	57	10
Dec.						
1	183	105	9	170	67	9
2	168(4)	97[4]	17	159(4)	65	19
3	172	104	11	164	70	11
4	172	110[5]	17	162	75[5]	16
5	166	114[5]	13	162(5)	77[4]	14
6	167(6)	112	17	157(6)	78	17
7	160(7)	97(5)[7]	7	155(7)	80	10
8	160(8)	100	18	154(8)	84[9]	18
9	160(9)	100	13	153(9)	86	17
10	168(10)	100	19	156(10)	89	18
11	164	104	20	158	95	17
12	166	107	23	159	103	26
13	174	117	21	164	109	26
14	172	120	19	168	112	23
15	172(11)	118	31	169(11)	113	23
16	175	123[10]	23	170(12)	112	20
17	166(13)	124	18	174	117	27
18	178	123	26	174	119	26
19	178	131	23	178	127	29
20	181	137	27	178	130	24
21	176	136	19	173	131	20

TABLE I (*Continued*)

Date (1927)	Female 12[1]	Litter	Food consumed during previous 24 hrs.	Female 13[1]	Litter	Food consumed during previous 24 hrs.
Dec. (*cont.*)	gm.	gm.	gm.	gm.	gm.	gm.
22	184	138	26	178	134	26
23	188	142	26	180	138	24
24	191	147	25	184(14)	139	29
25	190(15)	150	24	182	142	26
26	183(16)	151	23	186	147	29
27	183	158	23	184	155	27
28	188(17)	158[6]	23	187	160	30
29	182	160	21	187(18)	164	28
30	186(19)	162	24	188(19)	167	30
31	183(20)	163	27	188	172	37
Jan. 1928						
1	180(21)	166	24	185(21)	168	33
2	182	176	20	185	179	30
3	186	184	25	184	186	33
4	182	186	27	186	188	34
5	186	196	34	184	198	36

[1] Females 12 and 13 had litters of 11 and 6 young respectively and were each allowed 6 to rear.

[2] Composition of Ration 1009: casein (purified) 20.0, agar-agar 2.0, McCollum's Salt Mixture 185 4.0, butter fat 5.0, dextrin 69.0.

[3] Eyes open.

[4] All young dragging hind legs.

[5] Young having screaming running fits.

[6] Dragging hind legs slightly.

[7] Number in parenthesis indicates how many live young were left on that particular day.

[8] For method of preparation, see text. (Yeast Concentrate No. 18 corresponds to Lilly No. P-20308.)

[9] Young observed in feeding pan.

[10] Young having running fits.

(1) 25 mg. of Yeast Concentrate No. 18 to Female 13 daily.
(2) 50 mg. of Yeast Concentrate No. 18 to Female 13 daily.
(3) 50 mg. of Yeast Concentrate No. 18 to Female 12 and 13 daily.
(4) 75 mg. of Yeast Concentrate No. 18 to Female 12 and 13 daily.
(5) 27 mg. to Female 13 and 48 mg. to her litter daily.
(6) 21 mg. to both mothers and 54 mg. to both litters daily.
(7) 10 mg. to both mothers and 65 mg. to both litters daily.
(8) 35 mg. to both mothers and 40 mg. to both litters daily.
(9) 200 mg. to both mothers daily.
(10) 300 mg. to both mothers daily.
(11) 400 mg. to both mothers daily.
(12) 500 mg. to Female 13 daily.
(13) 500 mg. to both mothers daily.
(14) 10 mg. to Female 13 and 60 mg. to her litter daily.
(15) 40 mg. to Female 12 and 60 mg. to her litter daily.
(16) 28 mg. to Female 12 and 72 mg. to her litter daily.
(17) 16 mg. to Female 12 and 84 mg. to her litter daily.
(18) 10 mg. to Female 13 and 75 mg. to her litter daily.
(19) 10 mg. to both mothers and 90 mg. to both litters daily.
(20) 10 mg. to Female 12 and 96 mg. to her litter daily.
(21) 10 mg. to both mothers and 96 mg. to both litters daily.

was carried out on 23 mothers, and out of a total of 133 young allowed to be reared 129 were successfully weaned, a lactation efficiency index of 95.5 per cent, on a daily distribution of a maximum of 118 mg. between mother and young. (Tables I to III inclusive.)

The next step of procedure we attempted was to administer small amounts of yeast concentrate No. 18 to the lactating mothers before they exhausted their entire supplies of vitamin B reserves. The purpose of such a modification of the technique was to bring the young up to such a stage of development that we could proceed with vitamin therapy early in lactation. Lactation record of females 36 and 37, Table II, serves as an

TABLE II

LACTATION RECORD OF FEMALES 36 AND 37 (FIRST AND SECOND LITTERS) TRANSFERRED FROM STOCK COLONY NO. 1 TO VITAMIN B DEFICIENT RATION 1009

MINIMUM DAILY VITAMIN B REQUIREMENT FOR GROWTH OF NURSING YOUNG (YEAST CONCENTRATE NO. 18)[2]

Date (1927)	Female No. 36[1]	Litter	Food consumed during previous 24 hrs.	Female No. 37[1]	Litter	Food consumed during previous 24 hrs.
Nov.	gm.	gm.	gm.	gm.	gm.	gm.
28	279			289		
29	238	32		254	36	
30	237	34	12	249	41	5
Dec.						
1	248	36	15	262	48	9
2	238	44	11	244	52	18
3	226	48	9	241	58	10
4	213	54	14	235	63	16
5	210	62	11	237	68	16
6	205	68	15	228	74	16
7	202	76	17	224	78	16
8	196(1)	81	16	218(1)	82	17
9	196	90	19	218	86	15
10	196	99	18	221	93	16
11	195	106	22	218	98	18
12	194(2)	111	21	220(2)	101	25
13	190	120	18	217	107	15
14	190	124[3]	17	212	111[3]	13
15	189	129	22	218(3)	113	24
16	186	135	21	213	118	21
17	183(4)	136	18	209	121	18
18	184(5)	135	22	208(5)	125	21
19	185(6)	136	20	208	132	22

TABLE II (*Continued*)

LACTATION RECORD OF FEMALES 36 AND 37 (FIRST AND SECOND LITTERS) TRANSFERRED FROM STOCK COLONY NO. 1 TO VITAMIN B DEFICIENT RATION 1009

MINIMUM DAILY VITAMIN B REQUIREMENT FOR GROWTH OF NURSING YOUNG (YEAST CONCENTRATE NO. 18)[2]

Date (1927)	Female No. 36[1]	Litter	Food consumed during previous 24 hrs.	Female No. 37[1]	Litter	Food consumed during previous 24 hrs.
Dec.	gm	gm	gm	gm	gm	gm
20	182	150	30	204	144	32
21	176	164[7]	29	204	156[7]	30
22	180	172	34	204(7)	156	20
23	176(8)	172	26	202(8)	154	34
24	178(9)	172	22	199(9)	154	24
25	177	175	27	197	160	26
26	177	179	31	200(10)	160	24
27	172	183	26	194	166	24
28	173	186	25	190	170	34
29	172	192	30	193	180	32
30	174	198	32	192	186	30
31	174	202	38	188	190	34
Jan. 1928						
1	184(11)	202	40	179(11)	192	37
2	186	208	26	182	200	25
3	190(12)	204[3]	25	186	207	32
4	182	214[4]	37	193	223	44
5	180	220	36	198	228	62
6	182	230	39	201	240[6]	48
7	186	240[6]	46			

[1] Females 36 and 37 had litters of 10 young each and were each allowed 6 to rear.
[2] For method of preparation, see text.
[3] Young opened their eyes.
[4] No signs of paralysis.
[5] Incipient posterior paralysis.
[6] Young weaned.
[7] Young eating.
(1) 50 mg. of Yeast Concentrate No. 18 daily to both lactating females.
(2) 10 mg. to females and 40 mg. to litters daily.
(3) 10 mg. to Female 37 and 48 mg. to her litter daily.
(4) 10 mg. to Female 36 and 48 mg. to her litter daily.
(5) 10 mg. to both females and 54 mg. to both litters daily.
(6) 10 mg. to Female 36 and 60 mg. to her litter daily.
(7) 10 mg. to Female 37 and 70 mg. to her litter daily.
(8) 10 mg. to both females and 78 mg. to both litters daily.
(9) 10 mg. to both females and 90 mg. to both litters daily.
(10) 4 mg. to Female 37 and 96 mg. to her litter daily.
(11) 10 mg. to both females and 102 mg. to both litters daily.
(12) 4 mg. to Female 36 and 108 mg. to her litter daily.

example. After these females had four daily administrations of 50 mg. of yeast concentrate No. 18 and the nursing young weighed 17 to 18 grams each (still blind and constantly nursing) we could readily proceed with vitamin B administrations to the young with a medicine dropper. The daily dosages were then distributed between mothers and litters, allowing 10 mg. to the mothers and 40 mg. to the litters. Beginning with Dec. 16 the litter of female 36 showed maintenance for four days on a daily dosage to the litter of six of 40 to 54 mg., or 6.6 to 9.0 mg. per nursling. Note, however, the tremendous response when the daily allowance to the litter was increased to 60 mg., thus making available 10 mg. to each young (Dec. 25). During the subsequent three days the litter gained 36 grams. In other words, the addition of one milligram more daily resulted in such a radical change that complete maintenance was followed by marked growth, allowing an increase of 6 grams per young in only 72 hours. On a daily dosage of 10 mg. per nursling we have another maintenance curve for 3 days (Dec. 22-24 inclusive) and no response in growth is secured until the daily allowance to each nursling is increased to 15 mg. A further response is obtained when the daily administration to each young is increased to 17 mg., followed by a slight decline (Jan. 3). During the period of the daily increased vitamin dosages to the nursing young the lactating mother was receiving a maximum daily dosage of only 10 mg., and lost only 10 grams in weight in 22 days. On Jan. 3 the 112 mg. daily vitamin allowance for mother and litter was re-distributed, allowing a daily dosage of only 4 mg. to the mother and 108 mg. to the young, i.e., an increase of 6 mg. to the litter of 6, or an increase of just 1 mg. to each nursling per day, and the response was most remarkable. Instead of loss of weight we have rapid gains following and in 4 days subsequent to this re-distribution of the total vitamin daily allowance the litter was weaned.

The results with litter of female 37 are quite similar in the striking sensitiveness with which nursing young respond to slight increasing daily dosages of a vitamin B concentrate when no storage is allowed by giving only minimum daily allowance for continuous growth. A daily dosage of 9 mg. to 13 mg. to each young permitted maintenance only from Dec. 21 to Dec. 24 inclusive. An increase to 15 mg. daily to each nursling resulted in growth for a 24-hour period followed again by maintenance. On Dec. 26 the total daily dosage of 100 mg. was distributed between mother and young, allowing only 4 mg. to the mother and 96 mg. to the litter, i.e., only one additional milligram daily to each nursling. Such procedure was followed by appreciable growth during the subsequent 6 days, the litter of six gaining 32 grams. Since between Dec. 31 and Jan. 1

the mother lost 9 grams and her litter gained only 2 grams, 6 mg. was added to the daily allowance of the mother and 6 mg. to the daily portion of her litter, or one additional milligram per nursling per day. Such slight change in the daily vitamin therapy resulted in the gain of 22 grams of the mother and 48 grams of the litter in 5 days, and the litter was weaned

TABLE III

SUMMARY OF EXPERIMENTS ON THE MINIMUM DAILY REQUIREMENTS OF YEAST CONCENTRATE No. 18* FOR NURSING YOUNG, WEIGHING 30 GRAMS EACH, TO PERMIT A GAIN OF 10 GRAMS IN BODY WEIGHT

Litter of female (number)	No. young allowed to be reared	No. young weaned	Minimum daily dosage per nursling	Time required for each nursling weighing 30 grams to make 10 grams gain in body weight
			mg.	days
8	6	3	16.0	9
9	6	5	16.0	9
10	5	5	17.0	7
11	4	4	14.0	8
12	5	5	18.0	7
13	5	5	17.0	8
15	6	6	16.0	9
20	6	5	14.0	8
21	6	6	15.5	7
23	6	6	16.5	10
24	6	6	17.5	9
25	6	6	16.5	7
28	6	5	14.5	8
29	6	6	14.0	10
30	6	6	16.0	8
31	6	6	16.5	11
33	6	6	15.5	9
34	6	6	13.7	9
35	6	6	13.7	9
36	6	6	16.7	12
37	6	6	16.5	8
38	6	6	16.7	9
39	6	6	16.5	9
	Total: 133	127	Av.: 15.8	8.6

*For method of preparation, see text.

at the end of this last experimental period. It is quite clear, then, from Table II that nursing young are very sensitive toward their vitamin B requirements and that each nursling needs a specific amount of vitamin

B according to individual body weight and age (our experimental results show that weight is the primary and age a secondary factor) for continuous growth. We have found on some occasions when, on account of pressure of other work, we would be obliged to delay vitamin therapy until afternoon instead of at the regular hours in the morning, certain litters, receiving minimum dosages of vitamin B therapy, would develop convulsions, characterized by running fits accompanied by shrills which we relieved almost immediately with their delayed daily vitamin B therapy.

We have secured similar striking results, employing this newer technique of vitamin B distribution between lactating mother and nursing young, using yeast concentrate No. 18, on 21 more litters but space does not permit to present all of our findings in detail. We are, therefore, submitting a summary of that phase of our experiments during later stages of lactation showing the minimum daily requirements of the concentrate referred to above for nursing young weighing 30 grams each, in order to permit a gain of 10 grams in body weight, (Table III) since it is such daily data that will enable us to define the vitamin B unit of nursing young.

Since we found that nursing young are unable to partake of the maternal diet to any appreciable extent until they reach a body weight of 30 grams each, it was impossible for us to tell how much of a daily allowance of dehydrated yeast it is necessary to supply such baby rats until a litter of six reached a collective weight of about 180 grams. For this work we have employed the Federal dehydrated yeast already referred to in the text. While we have previously found that 16 mothers successfully weaned 88 out of 96 young on a daily dosage of 1500 mg., and 7 mothers on a daily dosage of only 1200 mg. of this Federal yeast, we encountered a few individuals that failed on a daily allowance of as much as 2000 mg. Female 64 (Table IV) is one of those mothers. Considerable progress was, however, made at the point of failure in lactation on a 2000 mg. daily dosage to the mother by a distribution of a total of 900 mg. of this yeast, allowing 150 mg. daily to the mother, and 750 mg. daily to the litter of six, or 125 mg. daily to each young. At that point each nursling weighed approximately 30 grams. Later it was necessary to increase the daily dosage to each young to 135 mg. and when each young approximated a weight of 40 grams it required 150 mg. of yeast daily for growth. The average daily minimum yeast requirement for each nursling of Female 64 to permit a gain of 10 grams in body weight is the average of 125 mg., 135 mg., and 150 mg., which is 136 mg.

TABLE IV

LACTATION RECORD OF FEMALES NOS. 64 AND 65 (FIRST LITTERS) TRANSFERRED FROM STOCK COLONY NO. 1 TO VITAMIN B DEFICIENT RATION 1009

MINIMUM DAILY REQUIREMENT OF A DEHYDRATED YEAST (FEDERAL)[6] FOR NURSING YOUNG DURING THE LATTER PART OF LACTATION

Date (1927)	Female No. 64[1]	Litter	Food consumed during previous 24 hrs.	Female No. 65[1]	Litter	Food consumed during previous 24 hrs.
Oct.	gm.	gm.	gm.	gm.	gm.	gm.
23	274			248		
24	200	34		194	34	
25	200	38	15	189	36	10
26	192	42	10	186	41	9
27	182	45	4	179	47	9
28	186	50	8	176	54	9
29	178	57	14	170	61	8
30	172	65	10	167	69	12
31	168	70	11	167	76	13
Nov.						
1	166	76	14	164	80	14
2	162	80	12	162	85	12
3	156	84	10	156(1)	86	16
4	154(2)	86	10	163	92	12
5	170(3)	87[8]	22	166(3)	99	22
6	173	96	23	168	108	19
7	168	102	20	170	115	24
8	172	111[9]	25	166	123	20
9	172	117	22	169	130[9]	22
10	176	126	24	173	137	25
11	174	134	24	172	146	22
12	177	138	30	172	126(5)[7]	28
13	182	147[9]	25	170	136	22
14	182	152	31	171	140[4]	26
15	178	160	28	170	144	28
16	180	166	28	172	148	22
17	180(4)	170	30	170(5)	157	22
18	184	174	32	174	156	26
19	194(6)	178	30	176(6)	152	20
20	173	183	32	190	157	30
21	180	190	28	172	165	28
22	181	194	36	168	168	24
23	184	200	36	170	172	32
24	183(7)	200	30	170	180	32
25	182	205	32	170	184	31
26	180(8)	214	31	172(8)	176	21
27	180	218	40	170	182	41
28	179	217	33	176	188	38
29	180	231	37	174(9)	181	36
30	187	244[10]	47	174	193	36
30				176	199[10]	38

In the case of the litter of Female 65 (which failed in lactation on a 1500 mg. daily dosage of yeast) we are dealing with 5 young and this litter received at a point when each young weighed about 30 grams a daily allowance of 150 mg., 162 mg., and 180 mg. of yeast, or an average of 164 mg. in order to make a gain of 10 grams in body weight.

Table V gives a summary of our results with 13 more litters, (the mothers of which failed during the latter part of lactation on a daily allowance of 1000 mg. to 1200 mg. of Federal yeast) showing the daily minimum requirements of the Federal dehydrated yeast, as a source of vitamin B of nursing young, weighing approximately 30 grams each, to permit a gain of 10 grams. The minimum for 88 young of the 15 litters varies between 120 mg. and 165 mg. per nursling and the time necessary to produce the 10 grams gain in weight in each young varies between 6 and 11 days. The average daily amount of yeast needed per 30 gram nursling to make the 10 gram gain in weight is 142 mg. Only one litter out of fifteen could make such gain in 6 days and only three litters required as long a period as 11 days. The average period is 8.2 days, and from results shown in Table V we may safely conclude that 7 to 10 days is the period in which we may expect a 30 gram nursling to make a gain of 10 grams, provided there is no previous vitamin B storage alowed to accumulate.

We are now ready to examine Table III, which shows a summary of results secured on 23 litters apportioned daily minimum amounts of yeast concentrate No. 18. It will be noted that the minimum daily dosage per 30 gram nursling required to produce a 10 gram gain in body weight varies between 13.7 mg. and 18.0 mg., there being only one litter that required a maximum of 18.0 mg. per young, and only four litters that needed a

FOOTNOTES FOR TABLE IV

[1] Females 64 and 65 had litters of 9 and 7 young respectively and were each allowed 6 to rear.
[2] Young opened their eyes.
[4] Young observed in feeding pan.
[5] This brand of "Federal" dehydrated yeast was furnished by Eli Lilly and Company, Indianapolis, Indiana.
[6] Young having muscular tremors and showing incipient posterior paralysis.
[7] Number in parenthesis indicates that one of the young died, leaving a total of 5 out of a litter of 6.
[8] Young eating.
[10] Young weaned.
(1) Female 65 given 1500 mgms. yeast daily.
(2) Female 64 given 1500 mgms. yeast daily.
(3) Female 64 given 1800 mgms. and Female 65 1500 mgms. yeast daily.
(4) Female 64 allowed 2000 mgms. yeast daily.
(5) Female 65 allowed 1800 mgms. yeast daily.
(6) Females 150 and litters 750 mgms. yeast daily.
(7) Females 64, 150, and her litter 810, mgms. yeast daily.
(8) Females 150 and litters 810 mgms. yeast daily.
(9) Female 65, 150, and her litter 900 mgms. yeast daily.

minimum of 13.7 to 14.0 mg. per young daily. All the rest of the litters show a daily requirement of 15.5 to 16.5 mg per young of this concentrate. The average for 133 young is 15.8 mg. The period required varies between 7 to 12 days, and since only one litter required 12 days and one litter 11

TABLE V

SUMMARY OF EXPERIMENTS ON MINIMUM DAILY REQUIREMENTS OF A DEHYDRATED YEAST (FEDERAL)[1] AS A SOURCE OF VITAMIN B FOR NURSING YOUNG, WEIGHING 30 GRAMS EACH, TO PERMIT A GAIN OF 10 GRAMS IN BODY WEIGHT

Litters of female (number)	No. young allowed to be reared	No. young weaned	Minimum daily dosage per nursling	Time required for each nursling weighing 30 grams to make 10 grams gain in body weight
			mgms.	days
12	5	4	150	7
13	6	5	120	9
18	6	6	125	7
62	6	6	125	6
63	6	5	138	10
64	6	6	136	11
65	5	5	156	10
69	6	6	150	11
72	6	5	165	10
73	6	5	150	8
76	6	6	130	8
88	6	6	150	6
89	6	6	150	11
91	6	6	163	9
70	6	6	123	10
	Total: 88	83	Av.: 142	8.2

[1] Furnished by the Eli Lilly and Company, Indianapolis, Ind.

days, it is concluded that the period is between 7 to 10 days. The average period is 8.6 days agreeing very closely with the average period found for the Federal dehydrated yeast, which is 8.2 days. *The vitamin B unit is*, therefore, defined as *the daily amount of vitamin B which must become available to a nursing young of the albino rat weighing 30 grams, in order to permit a gain of 10 grams in 7 to 10 days.* In determining the vitamin B content of any substance the maternal diet must be entirely deficient in vitamin B and adequate in all other respects and there must be no storage of vitamin in mother and young when the biological assay is begun. Also the mother must be allowed enough vitamin for maintenance only;

otherwise there is a possibility of her secreting daily small amounts of vitamin in the milk which would vitiate the minimum dosage required by the nursing young.

The vitamin B units of one gram of Federal dehydrated yeast and of one gram of yeast concentrate No. 18 can now be calculated. Since the average daily amount of Federal yeast required by a 30 gram nursling to gain 10 grams in 7 to 10 days is 142 mg., one gram contains 7.0 units. Similarly, since the average daily amount of yeast concentrate No. 18 required by a 30 gram nursling to make the same gain in the same period of time is 15.8 mg., one gram of this concentrate contains 63.3 units. In other words, concentrate No. 18 is nine times as concentrated as the yeast from which it was prepared.

The daily records of food consumption as well as the daily record of body weight serve as an additional aid in determining the vitamin requirement of the lactating mother. This is particularly true in the earlier periods of lactation before the nursing young are able to partake of the maternal diet. Considerable reduction in the daily food intake during that period of lactation is generally a safe index of insufficient vitamin allowance to the mother. If the vitamin B concentrate is potent enough so that one gram contains 60 units, 10 mg. daily to the mother is generally sufficient for maintenance. To date we have found 30 mg. daily to be the maximum for that purpose, when dealing with a concentrated preparation. After the young begin eating, the increase in food consumption is credited to the young and the total amount of food consumed is to a great extent proportionate to the amount of vitamin B made available to the young. Such phenomenon is quite apparent daily, and the daily food intake later in lactation serves, therefore, as an additional index of the vitamin B requirement of nursing young. We found on close observation that four or five days before weaning a good many litters became very lazy as evidenced by their constant desire to nurse when they were capable of receiving considerable supplementary nutrition by partaking of their maternal diet. We have, therefore, resorted to a practice in such cases to separate the litters from their mothers and proceed with collecting the same daily data on weight and food consumption, at the same time continuing with the distribution of vitamin between mother and young. Such procedure which we call "compulsory weaning" was generally followed by accelerated growth of the nursing young. We could by such technique determine the relative amounts of food consumed by mothers and young. Out of a total of 40 grams a litter of six consumed 28 and the mother 12 grams; in other

words, the litter consumes about 70 per cent of the total food intake during the very later stages of lactation.

We next proceeded to further concentrate vitamin B by adsorbing the product released from fuller's earth with alkali on silica gel and extracting the vitamin at various hydrogen ion concentrations, employing a modified method of Levene and van der Hoeven. (7) Because of lack of experience, we have lost considerable vitamin in our first attempt but have improved our yields appreciably in the second trial. We have also made another modification in the biological technique of assay which we adopted on our first concentrate prepared by the silica gel adsorption method. We began to administer small amounts of this concentrate to the mothers on the day of the birth of the litters. By drawing out a medicine dropper to a fine capillary we found that we could begin vitamin therapy as early as the fourth to the fifth day of lactation when each rat weighed 10 to 12 grams, or approximately one-third of an ounce. Employing such technique we found one gram of our first concentrate prepared by the silica gel adsorption method to contain 91.1 vitamin B units. The second concentrate prepared by the same method was considerably more potent, one gram containing 125 units.

We found our biological method so sensitive that, if during any stage of lactation when the young are receiving vitamin B therapy separately from the ration, a change is made in the character of the vitamin B concentrate, provided a potent preparation is introduced, definite and marked response is obtained in 24 hours. If such change is made during the later part of lactation when the nursing young are partaking of the maternal diet, the response is indicated not only by the increase of weight of the nurslings but also by the increase in the food intake.

We are now simplifying the technique for the determination of the vitamin B units in concentrated preparations, so that depletion of the vitamin reserves of the lactating mother takes place during the later part of lactation when the litter of six young collectively weigh 150 to 180 grams, or when each nursling weighs 25 to 30 grams. This will materially reduce the amount of routine labor and save considerable amounts of valuable vitamin B concentrates during the experimental period of biological assay. We expect to have such results ready for publication in the near future.

Summary

1. A quantitative biological method has been perfected for the vitamin

B requirements of nursing young which is sensitive to the extent of one milligram per nursling per day.

2. A vitamin B unit, applied to nursing young, has been defined as follows: The vitamin B unit is the daily amount of vitamin B which must become available to a nursing young of the albino rat weighing 30 grams, in order to permit a gain of 10 grams in 7 to 10 days.

3. One gram of a dehydrated baker's yeast, Federal brand, has been found to contain 7 units of vitamin B. Applying our quantitative biological method of assay to vitamin B concentrates we found variations to the extent that one gram contained as little as 14.4 units and as much as 125 units.

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VITAMIN REQUIREMENTS OF NURSING YOUNG

IV. VITAMIN B *VERSUS* VITAMINS A AND D REQUIREMENT FOR GROWTH OF NURSING YOUNG OF THE ALBINO RAT[1]

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FOR the past three years we have been conducting a series of experiments on the cod liver oil requirements for growth of nursing young, some of the results of which have already been reported. (1) In this paper we are submitting additional detailed evidence and a summary showing the average amount of cod liver oil,[2] as a source of vitamins A and D, necessary to administer to each nursling for continuous growth at the point of depletion of vitamin reserves of the lactating mother.

When females on the date of the birth of their litters are transferred from our stock diet No. 1,[3] which is a cereal ration containing one per cent cod liver oil, to our fat-soluble deficient ration 1034 (consisting of casein[4] 20%; salts 185, 4; Northwestern yeast, 10; and dextrin, 66) all the litters are reared and weaned at a normal rate without exception. (1) Such phenomenon we attributed to storage of vitamins A and D from the previous stock diet. In order to produce depletion of these fat-soluble vitamins, we were obliged to start another rat colony and we adopted the simplified diet of Sherman and Muhlfield (2) consisting of whole wheat 66%, whole milk powder 32.7, and NaCl 1.3. The latter diet introduces approximately 9 per cent milk fat which is now generally recognized to be considerably inferior in potency of vitamins A and D to cod liver oil, and on such a dietary regime we were able to produce depletion of vitamin

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[2] We have administered the same brand of cod liver oil employed by us since 1921.

[3] Composition of stock diet No. 1: whole wheat 27%, rolled oats 26, yellow corn 25, oil meal 15, commercial casein 5, cod liver oil 1, NaCl 0.5, $CaCO_3$ 0.5; and a liberal supply of milk daily.

[4] Hot alcohol extracted.

TABLE I

LACTATION RECORD OF FEMALE NO. 97 (FIRST LITTER, 7 YOUNG BORN, AND 6 ALLOWED TO BE REARED) TRANSFERRED FROM STOCK DIET NO. 2[1] (THIRD GENERATION) TO FAT-SOLUBLE VITAMIN DEFICIENT RATION 1034[2] VITAMINS A AND D (FURNISHED BY COD LIVER OIL) REQUIREMENTS OF NURSING YOUNG FOR GROWTH

Date (1927)	Female No. 97	Litter	Food consumed during previous 24 hrs.	REMARKS
Oct.	gm.	gm.	gm.	
28	237			[1] Composition of Stock Diet No. 2 :
29	170	32		Whole wheat.....66.0
30	167	30	9	Whole milk powder.....32.7
31	172	31	20	NaCl..... 1.3
Nov.				
1	170	36	19	[2] Composition of Ration 1034:
2	178	39	17	Casein (hot alcohol extracted) .20
3	179	47	22	Salts No. 185..... 4
4	181	54	25	Northwestern Yeast.....10
5	182	62	25	Dextrin.....66
6	178	71	21	
7	178	78	25	
8	181	86	23	[3] Young opened their eyes.
9	180	97	24	
10	183	103	22	[4] Number in parenthesis indicates that one young died, the total litter of 6 having been reduced to 5.
11	181	11	26	
12	180	19	24	
13	187	125[3]	26	
14	190	128	10	[5] One drop of cod liver oil (weighing 12 milligrams) to each young.
15	188	108[3]	8	
16	182	94[3]	12	
17	177	79(5)[4,3]	14	
18	196	86[3]	28	[6] Cod liver oil administration discontinued.
19	184	92	24	
20	186	94	26	
21	188	99	28	[7] Young eating.
22	189	108[7]	36	
23	190	114	28	[8] Young weaned.
24	190	124	36	
25	192	136	34	
26	194	146	32	
27	198	157	33	
28	194	165	40	
29	188	173	42	
30	193	184	35	
Dec.				
1	192	194	53	
2	192	202[8]	35	

reserves in mothers during lactation, so that nursing young responded readily to cod liver oil therapy. Such vitamin depletion we were not able to secure, however, until the second generation. In pursuing the method of transfer experiments with females and litters of the third generation we have encountered an entirely different situation. The mothers on fat-soluble deficient ration 1034 would dispose of their young during the first few days of the nursing period; so we were handicapped again in the continuation of the problem. About 120 young were lost in this manner that were not available for cod liver oil therapy, although considerable time and care were consumed in getting ready for such experimentation. Fortunately, however, a limited number of mothers did not deplete their vitamin reserves until later in lactation; also several mothers kept their litters during the first few days of the nursing period at a maintenance level. The latter performance of the lactating mothers gave us an opportunity to proceed with cod liver oil therapy to the nursing young.

Detailed records of several litters are shown in Tables I and II, and a summary of all of our work to date is presented in Table III.

Lactation record of Female 97 (Table I) shows appreciable loss of weight of the litter on Nov. 15. One drop of cod liver oil per nursling did not check the further decline of weight and another drop per nursling was then administered on Nov. 16. One young out of the litter of six died the next day. One drop of cod liver oil to each of the remaining 5 young on Nov. 17 produced subsequent continuous growth for weaning of the litter.

Lactation record of Female 98 (Table II) shows that her litter needed only one drop of cod liver oil per young as early as the third day of lactation but nothing more for continuous growth during the rest of the nursing period when administered at a point of abnormally slow growth indicating inability of the mother to secrete any more of her fat-soluble vitamin reserves in the milk.

In the case of lactation record of Female 99 (Table III) apparently six drops of cod liver oil, one for each young, administered to the mother at a point in lactation when the litter showed loss of body weight, were sufficient for rearing and weaning of the young. We have made similar observations on five additional mothers and it seems that the lactating mother is considerably more efficient in the transfer of the fat-soluble growth-promoting vitamins A and D (carried by cod liver oil) (4) than in the secretion of vitamin B (furnished by yeast concentrates or alcoholic extracts of the wheat embryo) (3) in the milk.

TABLE II

LACTATION RECORD OF FEMALES NOS. 98 AND 99 (FIRST LITTERS) TRANSFERRED FROM STOCK DIET NO. 2[4] (THIRD GENERATION) TO FAT-SOLUBLE VITAMIN DEFICIENT RATION 1034[4]

Date (1927)	Female No. 98[1]	Litter	Food consumed during previous 24 hrs.	Female No. 99[1]	Litter	Food consumed during previous 24 hrs.
Oct.	gm.	gm.	gm.	gm.	gm.	gm.
30	275			210		
31	207	30		155	30	
Nov.						
1	216	30	14	165	34	17
2	218	32	17	163	37	16
3	218	35[3]	17	168	43	23
4	222	40[3]	23	167	50	25
5	220	46	22	168	56	24
6	220	52	20	170	63	24
7	217	59	22	169	70	29
8	222	67	25	169	79	23
9	221	71	25	170	85	26
10	222	77	22	170	94	21
11	220	86	28	172	102	30
12	221	94	29	166	111	26
13	221	100	28	166	119	23
14	222	110	31	170	126	29
15	222	118	25	163	133	16
16	220	124	31	154(1)	130	16
17	216	128	30	161	127	23
18	218	134	36	160(2)	132	31
19	217	146	30	159	142	20
20	216	149	26	160	145	28
21	226	156[5]	42	166	151	32
22	216	158	38	166	166[6]	38
23	218	164	24	166	170	24
24	217	174	40	167	186	38
25	216	200[6]	52	165	202	44
26				170	210	36
27				170	223	38
28				171	240[6]	53

[1] Females 98 and 99 had litters of 5 and 6 young respectively and were allowed their total litters to rear.
[2] Cod liver oil administration discontinued.
[3] One drop of cod liver oil (weighing 12 milligrams) to each nursing young.
[4] For composition of rations, see Table I.
[5] Young eating
[6] Young weaned
(1) 6 drops of cod liver oil (each weighing 12 milligrams) to mother daily.
(2) Cod liver oil administrations to mother discontinued.

TABLE III

AMOUNT OF COD LIVER OIL AS A SOURCE OF VITAMINS A AND D NECESSARY FOR WEANING OF NURSING YOUNG WHEN ADMINISTERED TO THE NURSLINGS AT THE POINT OF DEPLETION OF FAT-SOLUBLE VITAMIN RESERVES OF THE LACTATING MOTHER

Litter of female number	First Administration			Second Administration			Third Administration			Fourth Administration			Total c. l. oil[1] to each nursling during lactation (mgms.)
	Age (days)	Wgt. of 6 young (gm.)	c. l. oil[1] per nursling (mgms.)	Age (days)	Wgt. of 6 young (gm.)	c. l. oil[1] per nursling (mgms.)	Age (days)	Wgt. of 6 young (gm.)	c. l. oil[1] per nursling (mgms.)	Age (days)	Wgt. of 6 young (gm.)	c. l. oil[1] per nursling (mgms.)	
72	9	85	12										12
31	10	54	12	26	125	18	27	129	18				48
32	3	35	12	21	155[2]	18	22	156[2]	18				48
33	3	30	12										12
44	16	125	9	17	124	9	18	126	18				36
45	3	31	12	6	41	12	7	47	12	28	183	18	54
97	17	108	12	18	94	12	19	79	12				36
98	3	35[2]	12										12
100	3	36[2]	12	7	58	12	17	128	12				36
106	2	30	12	3	30	12							24
48	2	25	12										12
													Average 30

[1] c. l. oil = Cod Liver Oil.

[2] weight of 5 young.

57 out of 64 young were successfully weaned on the cod liver oil dosages administered to the nurslings at the point of depletion of the reserves of fat-soluble vitamins A and D, as shown in the above table

It is evident from Table III that after the lactating mothers have depleted all their vitamin reserves, as is evidenced by the plateau curve of the nurslings, very little cod liver oil is required for the continuous growth of the nursing young for weaning. A minimum of 12 mg. and a maximum of 54 mg. were found necessary for the entire lactation period, or an average for 57 young of 30 mg. per nursling.

In addition to the experiments recorded in Table III we have transferred four mothers (allowed 24 young to be reared) from our regular stock diet No. 1 to ration 1034 irradiated for 30 minutes with a mercury quartz vapor lamp at a distance of 24 inches to insure an adequacy of vitamin D. The young then required only vitamin A for further growth. (4) The average requirement of cod liver oil by each nursling after cessation of growth during various periods of lactation we found to be 24 mg.

A careful analysis of the minimum requirements of yeast concentrate No. 18 per nursling presented in the preceding communication (5) has revealed to us data enabling us to make a comparison of the vitamin B versus cod liver oil requirements for growth of nursing young. A good many of the lactating mothers received large dosages of this concentrate for seven to ten days during lactation, so that the litters may have been securing an appreciable amount of vitamin B from the mother's milk. Such detailed results we cannot introduce in this comparative analysis, since what we want to know is how much total vitamin B, as furnished by concentrate No. 18, the young needed throughout lactation when the mother received only enough of this vitamin B concentrate for her own maintenance. Such information we were, however, able to obtain from nine litters, and a summary of our results is shown in Table IV. The total amount of yeast concentrate No. 18 given to each nursling during lactation for successful weaning varies from 275 mg. to 486 mg., the average figure being 366 mg. Since this concentrate is nine times as potent as the dehydrated yeast from which it was prepared, the equivalent amount of yeast needed by each nursing young during lactation as a source of vitamin B is 3294 mg. It will also be noted that the mothers of these litters needed an average of 750 mg. of the yeast concentrate during lactation for their own maintenance. Comparing the minimum requirement of yeast and cod liver oil (the two most potent sources of vitamin B and vitamins A and D respectively) for the growth of nursing young during lactation when administered at the point of depletion of the vitamin reserves of the lactating mother we have a figure of 3294 mg. for yeast and 30 mg. for cod liver oil; in other words, nursing young of the albino rat require a minimum of 108 times as much vitamin B, calculated in terms of de-

hydrated yeast, as vitamins A and D furnished by cod liver oil for continuous growth during the nursing period. Also the maximum number of administrations found necessary to administer cod liver oil to the nursing young during lactation is 4, while that of yeast concentrate No. 18 is 29. None of the mothers whose litters received cod liver oil needed vitamin

TABLE IV

TOTAL AMOUNT VITAMIN B CONCENTRATE NO. 18[1] NECESSARY TO SUPPLY NURSING YOUNG AND MOTHERS DURING LACTATION WHEN ADMINISTERED AT THE POINT OF DEPLETION OF VITAMIN RESERVES OF THE LACTATING MOTHER

Litter of Female (number)	Number of Administrations to Young	Total amount of vitamin given to each nursling during lactation for successful weaning (mgms.)	Number of Administrations to mother	Total amount of vitamin given to mother during lactation (mgms.)
29	23	275	32	942
30	29	357	37	714
31	33	450	41	772
32	25	371	35	1127
33	22	338	35	1183
36	27	347	31	440
37	26	332	30	424
38	42	486	43	652
39	31	342	35	498
Average:	29	366	35	750

[1] This yeast concentrate is nine times as potent as dehydrated yeast, Federal brand, furnished by Eli Lilly and Company, Indianapolis.

therapy for her own welfare. They evidently still had enough reserves for maternal welfare but insufficient storage so that the surplus could be secreted in the milk. On the other hand, the mothers of the litters receiving vitamin B therapy needed an average of 35 vitamin administrations for their own maintenance. When it is considered that there may be a number of brands of cod liver oil more potent than ours, the relative differences in the vitamin B and cod liver oil requirement for growth of nursing young may be considerably greater.

DISCUSSION

Our illuminating findings that nursing young need approximately 100 times as much vitamin B (calculated in terms of dehydrated yeast) as vitamins A and D (as furnished by a brand of cod liver oil employed by us since 1921) for growth during the nursing period brings up the question:

Do infants, breast or artificially fed, receive adequate amounts of vitamin B?

In 1916 Eddy and Roper (6) demonstrated the stimulation of growth in marasmic infants by the introduction of a powder prepared from the alcoholic extract of the pancreatic gland of lambs into the diluted milk formulae of such athreptic babies. In 1922 Daniels (7) has shown similar responses in growth secured on artificially fed infants by the addition of alcoholic extracts of the wheat embryo to the daily diet of the infants. The recent quantitative work of Macy, Outhouse, Graham, and Long (8) show that pooled human breast milk taken from individuals subsisting on the average American diet is markedly deficient in vitamin B and these investigators suggest that every infant, breast or artificially fed, should receive some supplementary vitamin B product early in the nursing period.

Of all the disturbances responsible for infant mortality during the first year, perhaps none of those known to medical science causes greater apprehension than diarrhea and enteritis, since 20 to 30 per cent of infants die yearly from gastro-intestinal diseases, (9) the etiology of which is apparently not as yet clearly understood, as is evidenced from the following statement by Abt in his "Pediatrics" (10):

> "In attempting to write a chapter on the nutritional disturbances of infancy, one is confronted with the fact that, while an immense amount of work has been done, the theories are conflicting and there are many gaps in our knowledge. From one period to another views have changed concerning etiologic factors in nutritional disturbances. The ink is scarcely dry on the printed page before some new publication controverts what has previously been written. For this reason dogmatic statements concerning etiology are not possible at present."

Since the maternal diet and the artificial feeding of infants can never be completely and rigidly controlled by the physician, particularly from the standpoint of depleting vitamin reserves, in order to establish the minimum requirement of such dietary components for adequate nutrition, problems of infant mortality as related to the diet must necessarily be investigated first on small experimental animals. I have been carrying on such investigations on the albino rat since 1919, and after eight years of continuous failure I have at last succeeded in 1927 in inducing lactating rats to rear and wean their young on synthetic diets composed of purified food substances. This was accomplished only after increasing the vitamin B content of the diet to unusually large proportions (3). In pursuing the problem further it was discovered that the lactating mother wastes at least 60 per cent, if not more, of the total vitamin B daily apportioned to her in the metabolism of transfer to the milk, and that while the young are dying when the maternal diet is inadequate in vitamin B for lactation the mother is

storing that vitamin in her tissues. It was also found that the nursing young themselves respond most markedly to vitamin B administrations. (11) During the various stages of vitamin B deficiency we have to date found the following pathological symptoms:

I. Gastric stasis (12.) The young die with their stomachs well distended with curd, so the infant mortality must be due to poor quality rather than to insufficient quantity of milk elaborated by the mother.

II. Paralysis, extending from the posterior quarters to the jaws and pharynx, so that the nursing young in the spastic condition are unable to swallow readily.

III. Muscular tremors.

IV. Convulsions, characterized by screaming running fits so the nurslings bite the screens until they bleed from the mouth.

V. Hemorrhages in bones, particularly at the juncture of the occipitals and parietals (12).

VI. Hemorrhages in viscera, i.e., spleen, kidney, thymus, and in the intestinal tract (12).

VII. The intestinal tract is frequently found filled with gas (13).

VIII. Fatty degeneration of the liver, characterized by extreme vacuolation, capillary congestion, and edematous condition (13).

IX. Marked anhydremia associated with disturbance in hematopoietic function. The concentration of the blood is 25 to 40 per cent, and the color index above one (14). We are at present producing rapid blood regeneration employing one of our potent yeast concentrates.

X. Hypoglycemia, i.e., a drop from 110 mg to 80 mg per 100 cc of blood, and restoration of the normal sugar level in the blood by vitamin therapy (15).

XI. During the last stage of vitamin B deficiency the nursing young develop cyanosis and respiratory failure.

The most remarkable thing about the condition of the nursing young suffering from vitamin B deficiency is that they respond most readily to vitamin B therapy after growth has completely ceased, in the convulsive state, and in a condition when their swallowing centers are paralyzed. The response is obtained within a few minutes. They become perfectly restful and are able to swallow readily after a few drops of a concentrated vitamin B preparation are forced down by means of a medicine dropper.

Recently we have developed on a modified dietary regime (16) a new condition in the nursing young, i.e., prolonged maintenance of body weight unaccompanied by any apparent external pathological symptoms, and in such condition the young show marked pathological changes in the liver;

anhydremia, anemia and hypoglycemia; yet in such pathological state the nursing young respond most readily to vitamin therapy as evidenced by rapid increases in growth and food consumption, and rapid regeneration of blood accompanied by restoration of the normal blood sugar level (15).

On the basis of our findings we feel justified in re-emphasizing the suggestion made in previous publications, namely, that a great proportion of the infant mortality during the first year of life, associated with gastro-intestinal disturbances, may be attributable to a deficiency of the vitamin B complex in the diet of the nursing mother, especially the American woman, and also to her physiological inability to secrete that vitamin complex quantitatively in the milk. (11)

During the past decade a considerable literature has accumulated on the etiology of rickets and scurvy. Pediatricians already recognize the advantages of the prophylactic method and, hence, recommend as early as the fourth week of life orange or tomato juice as a source of vitamin C and cod liver oil to supply the necessary needs of vitamins A and D. No provision is as yet, however, being made for prophylactic or curative vitamin B therapy.

Summary

Nursing young need approximately 100 times as much vitamin B (calculated in terms of dehydrated baker's yeast) as vitamins A and D (as furnished by a brand of cod liver oil employed by us since 1921) for continuous growth during the nursing period.

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SOME FACTORS AFFECTING THE CONNECTIVE TISSUE CONTENT OF BEEF MUSCLE

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IT IS an encouraging sign that investigators in meat production are diverting an increasing proportion of their time and energies from the factors affecting the economy of meat production to those affecting the quality of the meat produced, from the standpoint of its desirability to the consumer. It is a matter of common experience that different cuts of meat differ decidedly in tenderness, juiciness, and flavor, and particularly that the same cuts from different carcasses also show decided differences in these respects. A study of the factors affecting the quality and palatability of meat, as they relate to the selection of animals for meat production, to their feeding and management, and to the preparation of the meat for consumption, should ultimately determine to what extent the quality and palatability of meat can be controlled, and what methods of selecting, managing and feeding animals will assure the production of the most desirable kinds of meat. A national cooperative project among the state agricultural experiment stations and the United States Department of Agriculture concerned with these problems has been under way for a number of years, and the investigation to be reported in this paper is an integral part of this national project.

The quality of a meat carcass is judged largely by its conformation, by the amount, color, firmness, and distribution of its fat, and by the color, texture and firmness of its lean. To the consumer, however, the quality of the *lean* meat is the paramount consideration. It is true that the amount of fat surrounding the lean in a retail cut of meat modifies its palatability after cooking, but it is commonly considered that most of this separable fat is a table waste. To a certain extent, it is an unavoidable waste in so far as it is a necessary accompaniment to the deposition of fat (marbling) within the lean, one of the conditions of quality in meat.

Since it is the lean meat that the consumer buys primarily, the quality of lean meat should be the primary object of study. It may be questioned whether and to what extent the color, texture, and firmness of lean meat

contribute to its palatability, but there is no question that tenderness, juiciness, and flavor are important properties in this respect.

The scientific study of these properties must obviously be preceded by the devising of satisfactory methods of measurement.

We have concerned ourselves with the property of tenderness, or its converse, toughness. The mechanical methods of measuring toughness that have been proposed and tried are subject to two difficulties, one the purely instrumental one of devising a machine that will be sensitive to small differences in toughness and that will give reproducible values for the same cross-section of tissue, and the other the difficulty of securing proper samples, and representative cross-sections. The first difficulty may ultimately be solved, but the second is inherent in the principle of mechanical testing, which is concerned with pieces of tissue of considerable size whose structure has not been altered by the method of removal from the cut of meat from which it is taken. The preparation of a representative sample of a cut of meat in the chemical sense would render it unsuitable for mechanical testing. It would seem that this difficulty of sampling is a most serious one, to which the variation among results obtained on different portions of the same cut of meat may largely be traceable.

Lean meat is essentially muscle tissue, but it contains also considerable and variable amounts of connective tissue. According to the observations of Lehmann (1), it is the connective tissue fibers, rather than the muscle fibers, to which the greater portion of the toughness of meat is due. Since connective tissue fibers consist of two proteins, collagen and elastin, possessing more or less characteristic chemical properties, a basis for a chemical determination of connective tissue is at hand; and, in so far as connective tissue accounts for the toughness of meat, the results obtained will be correlated with this important physical property. The errors of such a determination are largely errors in the chemical separations attempted, since the error of sampling can be largely eliminated by the proper comminution and mixing of the material analyzed.

Analytical Method Used

A chemical method for the determination of the collagen and elastin in lean meat has been worked out in this laboratory, and in its first form it has been published (2). Continued use of the method has suggested improvements, particularly with regard to the final separation of elastin from the muscle proteins by enzyme digestion. In the original description of the method, the enzyme digest was boiled before it was filtered. However, it appears that intermediate hydrolytic products of the muscle pro-

teins are thrown out of solution by this procedure and contaminate the elastin. Our published determinations of elastin in muscle meat are probably, for this reason, all of them too high.

Other changes of less importance have also been made. If the first washing and sieving of the macerated meat is done with water at a temperature of 45° to 50°C., instead of with cold water, the fat will interfere less. A 100-mesh sieve has been used in this first process and also in the final washing of the elastin. The gelatin filtration is now made with a linen instead of a paper filter. A complete description of the revised method follows:

Free the meat from all visible fat and surrounding connective tissue. Grind the sample through a meat chopper using a medium cutting plate. All meat remaining in the mill should be removed completely, chopped up, and thoroughly mixed with the ground sample. Duplicate samples of 100 gms. each may be used. Place each sample in a small ball mill[1] with 300 cc. of distilled water and macerate for 90 minutes. Transfer the sample onto a 100-mesh sieve, rejecting the filtrate. Wash the residue into a casserole with 100–150 cc. of water at 45° to 50°C., stir thoroughly, and filter through the 100-mesh sieve. Repeated tests have shown that seven such washings are sufficient to remove practically all of the water-soluble constituents of the meat and a good deal of the finely divided granular material.

Transfer the residue from the sieve to an 800 cc. beaker with hot water. Bring the volume up to about 400 cc., add a few small pieces of porous porcelain, and heat for 2 hours in an autoclave under 15 pounds steam pressure. Release the pressure in the autoclave gradually (taking about 30 minutes) and do not disturb the beakers for at least 5 minutes after the door is opened, for as long as the contents of the beakers are superheated there is danger of loss. Decant the hot supernatant liquid through a linen filter onto a Büchner funnel, collecting the filtrate in a suction flask. No suction is applied, however. Wash the residue on the filter into a mortar with a few cc. of hot water, and macerate gently with a pestle until the lumps of coagulated protein are broken up. Transfer to the beaker with about 100 cc. of hot water, boil for a few minutes, stirring constantly to prevent bumping, and filter again. The washing with boiling water is repeated five times, or until the washings give only a constant faint color with the biuret test. Make the combined filtrates up to 1 liter

[1] We have used for this purpose a motor driven ball mill (Fisher Scientific Co. No. 8-380) holding two porcelain jars. In our experience, the best results have been obtained by the use of 12 steel balls in each jar, 6 having a diameter of 1.5 inches and 6 a diameter of 1.0 inch.

in a volumetric flask and take one-tenth aliquots for total nitrogen determinations.

Wash the residue on the linen back into the 800 cc. beaker with 200 cc. of cold trypsin solution.[2] Add 3 cc. of a mixture of equal parts of chloroform and toluene and digest for exactly 16 hours in a constant temperature oven at 38° to 40°C. The digest should be brought to 40°C. before being put in the oven. At the end of the 16 hours, filter the trypsin digest through a 100-mesh sieve, washing four or five times with water at about the same temperature (35° to 40°C.) until all material that will go through the sieve has been washed out of the residue. The residue is then transferred to a mortar with as little water as possible, broken up with a pestle, and then washed repeatedly on the sieve until only small white thread-like fibers remain. The filtrates and washings are rejected. The residue on the sieve is transferred to a Kjeldahl flask, digested in the usual manner and distilled either directly, or by aliquot if the amount of residue is large.

The collagen and elastin nitrogen as thus determined may be expressed as percentages of the total nitrogen in the meat, or as percentages of collagen and elastin in the fresh meat, taking the nitrogen content of collagen as 17.90 per cent and that of elastin as 16.87 per cent (3).

Considerable attention has been given to the proper length of time to heat the meat samples in the autoclave for the conversion of the collagen contained in them into gelatin. Periods of one-half hour and of one hour were definitely shown to be insufficient, even with the subsequent boiling and washing prescribed in the method. A period of one hour and a half seemed to be long enough for the samples of meat tested (shoulder and fore shank), but a two-hour period of heating was finally decided upon.

It is realized that this is not a precise chemical method, since it is based upon separations that cannot be considered quantitative. Some idea of the reproducibility of the results obtained may be secured by our experience with the analysis of 183 samples of meat of all descriptions. When the collagen results are expressed as collagen nitrogen in per cent of the total nitrogen, the average difference between the 183 duplicates was 0.50, 56 per cent being less than this value, 63 per cent less than 0.60, 73 per cent less than 0.70, 77 per cent less than 0.80, 82 per cent less than 0.90, and 88 per cent less than 1.00. Since the probable error of the mean of duplicate determinations may be taken as half the difference between them (4), it

[2] The trypsin solution is made as follows: 150 mgms. of powdered trypsin (we use a product put out by Fairchild Brothers and Foster of New York), 20 cc. of a 3 per cent Na_2CO_3 solution, and 180 cc. of water. This solution is made up fresh each day.

follows that the average probable error of the means of duplicate values for collagen nitrogen obtained by this method is ± 0.25. This discussion refers to unselected analyses. In actual practice, the duplicates deviating the most widely will be discarded and the determinations repeated. In such a case, the average deviation between duplicates may be made smaller, and the distribution narrower than is indicated above. An insufficient number of elastin determinations have been made by the revised method to permit a significant analysis of their accuracy.

The practical value of this method of determining the characteristic connective tissue proteins in meat, relates more especially to the collagen determination, since in most cuts of meat the elastin occurs in only insignificant amounts. Possibly the accuracy of the collagen determination may be increased by salting out traces of muscle proteins that may contaminate the gelatin solutions, in accordance with the procedure of Striegel (5) in the analysis of commercial feeds for gelatin.

Our experience in applying this method to cooked meats does not warrant recommending its application in an unmodified form.

A Comparison of Heifer and Steer Beef

The material for this comparison was obtained from a feeding experiment being conducted by the Beef Cattle Division involving a comparison of heifer and steer calves in the production of baby beef. The two groups of calves from which animals were taken at different times were obtained from the same ranch in Texas and were quite uniform in breeding (largely Hereford blood) and age and were fed during the experiment upon the same ration of shelled corn, corn silage, cottonseed meal, and alfalfa hay. One heifer and one steer calf were slaughtered at the start of the experiment, and also after 140 and 200 days of feeding. Sixty-six days later, two steer calves were slaughtered for examination to permit a further study of the effect of age on the quality of lean meat.[3]

Eight retail cuts were taken for examination, and collagen and elastin determinations were made upon the lean of each. In Tables I and II, the results of the collagen determinations have been given in full, while only those elastin determinations have been reported that were made by the improved method, in which the boiling of the trypsin digest previous to filtration was omitted, thus permitting a visibly clear separation of the elastin from the muscle proteins.

[3] One of these steer calves (No. 72) received a ration of ear corn silage, cottonseed meal and alfalfa hay.

A study of Table I reveals no consistent differences between steer and heifer calves relative to the collagen content of the lean, while the elastin values in Table II are of like significance. However, among the different retail cuts, interesting and consistent differences appear. Considering the nitrogen percentages, the "ribeye" (the longissimus dorsi muscle) of the 9th, 10th and 11th ribs possessed, for practically all calves, the lowest

TABLE I

THE COLLAGEN CONTENT OF THE LEAN OF DIFFERENT CUTS OF BEEF TAKEN FROM STEER AND HEIFER CALVES

Calf No. and sex	47H[3]	84S	100H	41S	63H	51S	85S	72S
Body weight, lbs.	400	375	670	710	815	900	890	900
Days on feed	0	0	140	140	200	200	266	266
COLLAGEN NITROGEN IN PERCENT OF TOTAL NITROGEN								
Round steak	8.4	8.0	11.2	11.0	10.8	12.7	12.4	10.3
Porterhouse steak[1]	—	8.1	10.9	11.5	12.6	12.0	10.8	10.7
Sirloin steak[2]	8.3	8.8	8.3	9.8	13.3	12.4	11.5	11.9
Ribs (9, 10, 11) eye	8.8	8.4	7.2	9.6	8.9	8.7	7.2	6.8
Chuck ribs (2, 3)	11.3	14.9	13.6	13.9	15.6	14.8	17.0	16.1
Fore shank	24.4	22.3	22.2	24.7	23.7	22.0	24.7	20.2
Navel	16.7	14.8	18.0	16.9	14.1	15.7	15.7	14.9
Tenderloin	9.9	8.7	9.6	10.6	9.6	8.2	7.6	7.2
COLLAGEN IN PERCENT OF TOTAL FRESH LEAN								
Round steak	1.62	1.63	2.17	2.22	2.04	2.41	2.33	1.94
Porterhouse steak[1]	—	1.61	2.17	2.19	2.32	2.07	1.96	1.98
Sirloin steak[2]	1.63	1.64	1.64	1.91	2.50	2.40	2.07	2.16
Ribs (9, 10, 11), eye	1.72	1.76	1.43	1.91	1.74	1.64	1.33	1.27
Chuck ribs (2, 3)	2.06	2.76	2.42	2.51	2.65	2.52	2.78	2.76
Fore shank	4.65	4.43	4.27	4.67	4.43	4.20	4.52	3.87
Navel	3.05	2.88	3.05	3.02	2.16	2.52	2.36	2.54
Tenderloin	1.87	1.70	1.88	2.05	1.64	1.51	1.33	1.30

[1] The top muscle of the porterhouse only.
[2] The round-bone sirloin.
[3] H means heifer and S means steer.

content of collagen, with the tenderloin only slightly higher. The round, porterhouse, and sirloin were next in order, and are indistinguishable from one another in this respect. The chuck ribs and the navel contained consistently larger percentages of collagen nitrogen than the more expensive cuts just considered, but the fore shanks throughout all calves contained

the highest, the collagen nitrogen of this cut averaging 23 per cent of the total nitrogen of the lean.

The elastin percentages of Table II indicate a different distribution of this protein among the different cuts than the distribution of collagen. The tenderloin, sirloin and "ribeye" contain the lowest percentages of elastin, with the porterhouse and the fore shank next in order. The chuck ribs (2nd and 3rd) and round contain three times as much elastin as the porterhouse and fore shank, while the navel easily tops the list of all cuts in this respect.

TABLE II

THE ELASTIN CONTENT OF THE LEAN OF DIFFERENT CUTS OF BEEF TAKEN FROM STEER AND HEIFER CALVES

Calf No. and sex	63H	51S	85S	72S
Body weight, lbs.	815	900	890	900
Days on feed	200	200	266	266
	ELASTIN NITROGEN IN PERCENT OF TOTAL NITROGEN			
Round Steak	.35	.15	.25	.48
Porterhouse steak[1]	.08	.05	.09	.11
Sirloin steak[2]	.02	.02	.05	.06
Ribs (9, 10, 11), eye	.02	.03	.03	.05
Chuck ribs (2, 3)	.40	.26	.29	.24
Fore shank	.10	.10	.07	.10
Navel	.74	1.00	1.42	.92
Tenderloin	.07	.07	.02	.04

[1] The top muscle of the porterhouse only.
[2] The round-bone sirloin

Besides the two calves of each killing used for an examination of the eight chosen retail cuts, a number of other calves were taken for an examination only of the central muscle ("eye") from the 9th, 10th and 11th ribs. The collagen and elastin contents of the "ribeye" from these calves, as well as from those already considered, are summarized in Table III. Again no distinction between the lean meat of heifer and of steer calves is evident.

THE EFFECT OF AGE

The data summarized in Table I permit a comparison of the collagen content of beef cuts from calves of different ages. It is evident from a study of this table that a general statement on this point is not warranted. The meat from the youngest calves contained the lowest percentages of collagen nitrogen in the case of the round and porterhouse only. From the second to the third and fourth group of calves, the collagen content of the

sirloin has increased, and possibly that of the chuck ribs also. In fact, in the average, the chuck ribs increased in collagen content progressively from the first through the last killing. On the other hand, the "ribeyes" and tenderloins from the last group of calves were lower in collagen than those of the preceding groups. In the larger groups of calves also, the collagen content of the "ribeyes" of the oldest calves is perceptibly less than that of the preceding groups (see Table III).

TABLE III

THE COLLAGEN AND ELASTIN CONTENT OF THE "RIBEYE" FROM THE 9TH, 10TH AND 11TH RIBS OF HEIFER AND STEER CALVES

Days on feed	0	140	200	266
Approximate body wt., lbs.	400	700	850	850
	COLLAGEN NITROGEN IN PER CENT OF TOTAL NITROGEN			
Steer calves	8.4	9.6	8.7	7.2[1]
	8.1	7.1	8.6	5.4[1]
		7.8	9.0	6.4[1]
			5.9	6.8[2]
				5.4[2]
				6.0[2]
Heifer calves	8.8	7.2	8.9	
	11.7	7.4	9.5	
		8.2	9.2	
		8.5	7.7	
		9.2	8.4	
	ELASTIN NITROGEN IN PER CENT OF TOTAL NITROGEN			
Steer calves	—	—	.02	.02[1]
	—	—	.03	.06[1]
		—	.03	.02[1]
			.02	.05[2]
				.02[2]
				.06[2]
Heifer calves	—	—	.02	
	—	—	.04	
		—	.04	
		—	.05	
		—	.03	

[1] These steers received the same ration fed to the calves of all preceding groups, i. e., shelled corn, corn silage, cottonseed meal and alfalfa hay.

[2] These steers were fed a ration of ear corn silage, cottonseed meal, and alfalfa hay.

The results of a repetition of the above experiment with the same kind of calves except that all were steers, and the same ration, are given in Table IV. In this experiment only the "eye" muscle from the 9th, 10th and 11th ribs was analyzed. The five groups of calves of two each show quite irregular percentages of collagen nitrogen in the lean of the "ribeye," bearing no obvious relation to age, grading of carcass, or fat content of the lean.

TABLE IV

THE COLLAGEN AND ELASTIN CONTENT OF THE "RIBEYES" OF THE 9TH, 10TH AND 11TH RIBS OF STEER BEEVES OF DIFFERENT AGE AND GRADE

Steer No.	Length of feeding period	Body weight	Carcass grade	Dry matter content	Nitrogen content	Fat content	Collagen content	Elastin content	Collagen N in % of total N	Elastin N in % of total N
	days	lbs.		%	%	%	%	%	%	%
52	0	440	Medium	23.35	3.26	2.58	1.18	.046	6.5	.24
88	0	440	Medium	23.81	3.36	2.08	2.02	.036	10.8	.18
24	114	650	Good	26.31	3.59	1.53	1.15	.005	5.8	.02
94	114	680	Choice−	25.74	3.51	2.61	1.00	.012	5.1	.06
87	149	770	Good+	26.31	3.59	3.00	2.25	.006	11.2	.03
89	149	780	Choice−	27.25	3.44	5.25	1.96	.015	10.2	.07
P17	184	770	Good−	27.61	3.62	4.05	.97	.003	4.8	.01
69	184	820	Choice	29.00	3.65	4.78	1.24	.002	6.1	.01
P16	213	930	Choice+	30.04	3.23	8.21	1.54	.003	8.6	.01
65	213	975	Choice	29.05	3.40	6.64	1.58	.006	8.4	.03

The Effect of Condition (Fatness or Market Finish)

The fitness of a meat animal for slaughter is largely judged by its fatness. It is a question of great practical importance, therefore, whether this criterion for judging cattle and for assessing the value of their carcasses assures the best quality of meat from the consumer's standpoint. In particular, is quality of carcass (according to the butcher's grading) correlated with the connective tissue content of the lean meat—a dominant factor in its toughness?

In attempting to throw light upon this question, a number of rib cuts were obtained at local butcher shops from carcasses of known grading, and other rib cuts were obtained from experimental cattle slaughtered by the Meat Division at the University. The carcasses represented varied in grading from "choice" to "canner." The "ribeye" muscles only were submitted to analysis with the results given in Table V, arranged in the

Table V

The Collagen and Elastin Content of the "Ribeyes" of the 9th, 10th and 11th Ribs of Beeves of Different Grade

Carcass grade	Estimated age	Sex	Dry matter content	Nitrogen content	Fat content	Collagen content	Elastin content	Collagen N in % of total N	Elastin N in % of total N
	yrs.		%	%	%	%	%	%	%
Choice	1	Steer	25.82	3.59	2.50	1.08	.004	5.4	.02
Choice	2	Steer	27.87	3.55	4.35	1.89	.006	9.4	.03
Good+	4	Steer	29.07	3.25	7.78	1.19	.011	6.5	.06
Good	Young	Cow	25.16	3.42	2.99	1.16	.001	6.1	.005
Good	4	Steer	29.48	3.30	7.14	1.33	.002	7.2	.01
Good−	2	Steer	26.08	3.57	3.54	1.49	.003	7.4	.015
Medium+	1	Heifer	23.82	3.34	.97	1.05	—	5.6	—
Medium+	—	Cow	26.82	3.34	4.63	1.59	.002	8.5	.01
Medium+	3–4	Steer	26.83	3.36	5.18	1.32	.008	7.0	.04
Medium	3–4	Steer	26.12	3.40	3.62	1.15	.032	6.1	.16
Common+	Old	Cow	25.22	3.46	2.92	1.74	.004	9.0	.02
Canner	14–15	Cow	26.62	3.56	3.51	.92	.008	4.6	.04

order of decreasing grading. Evidently no correlation between grade of carcass and content of connective tissue in the "ribeye" is indicated by these data. In fact, the smallest percentage of collagen was found in the meat from a canner cow, the lowest grade of carcass, while the highest percentage was found in the meat from a choice two-year-old steer. However, the samples in between show such irregularities that this apparent

TABLE VI

THE COLLAGEN AND ELASTIN CONTENT OF THE INNER AND OUTER ROUND OF BEEVES OF DIFFERENT GRADE

Carcass grade	Estimated age	Sex	Part of round	Dry matter content	Nitrogen content	Fat content	Collagen content	Elastin content	Collagen N in % of total N	Elastin N in % of total N
	yrs.			%	%	%	%	%	%	%
Choice−	1	Steer	Inner	26.47	3.44	3.56	—	.010	—	.05
			Outer	25.97	3.42	2.54	1.46	.031	7.7	.16
Good+	3–4	Steer	Inner	28.75	3.09	8.04	2.29	.030	13.3	.17
			Outer	26.96	3.30	4.21	1.53	.003	8.3	.01
Good	1	Steer	Inner	26.68	3.63	2.26	1.33	.007	6.5	.03
			Outer	25.39	3.46	2.09	1.40	.077	7.2	.38
Good	2	Steer	Inner	27.26	3.67	3.35	1.85	.011	9.0	.05
			Outer	27.23	3.56	3.64	2.48	.055	12.5	.26
Good	3–4	Steer	Inner	30.55	3.02	9.79	2.17	.084	12.8	.47
			Outer	27.60	3.25	5.55	1.78	.005	9.8	.02
Medium+	5–6	Cow	Inner	27.37	3.70	3.57	2.08	.009	10.1	.04
			Outer	28.61	3.47	6.10	2.77	.102	14.3	.50
Medium+	3–4	Steer	Inner	26.07	3.51	3.49	1.29	.019	6.6	.09
			Outer	27.89	3.26	6.11	2.08	.189	11.4	.98
Medium	3–4	Steer	Inner	26.61	3.43	3.16	—	.012	—	.06
			Outer	28.13	3.20	6.42	1.82	.111	10.1	.59
Medium	2	Steer	Inner	26.28	3.75	2.03	2.10	.007	10.0	.03
			Outer	26.30	3.56	2.57	2.24	.047	11.3	.23
Medium	6–8	Cow	Inner	26.21	3.61	3.13	2.20	.008	10.9	.04
			Outer	25.85	3.55	2.58	2.71	.030	13.7	.14
Common	6–7	Cow	Inner	26.95	3.80	2.71	2.18	.020	10.3	.09
			Outer	26.44	3.59	3.06	2.45	.038	12.2	.18
———	Mature	Bull	Inner	25.48	3.65	1.13	2.16	.004	10.6	.02
			Outer	24.81	3.63	0.69	2.54	.036	12.6	.17

inverse relation is insignificant. It is of interest also to note that there is no consistent relation evident between the carcass grading and the fat (ether extract) content of the "ribeye."

The study of the relation between the grade of carcass and the connective tissue content of its lean was considered of such practical importance as to warrant an extension to other cuts of meat. Consequently a series of round steaks, taken from approximately the same part of the round from carcasses of known grading, were obtained either at local butcher shops, or from the carcasses of experimental cattle at the University. The inner and outer muscles of each round steak were analyzed separately. The results obtained are given in Table VI, and are arranged in the order of decreasing carcass grading.

Again there appears to be no relation between the grading of a carcass and the connective tissue content of its lean. Nor is there any relation between the carcass grade and the fat content of the lean. However, a fairly consistent relation exists between the inner and outer muscles of the round. Among the twelve rounds examined, the inner muscles (on the inside of the leg) contained much the smaller percentages of elastin in nine, and smaller percentages of collagen in seven, the comparison of collagen percentages being impossible in two cases because of unsatisfactory determinations in two samples of inner round. On the basis of the elastin results in particular, it appears not improbable that in the two exceptional rounds, in which the inner muscles contained *by far the larger* percentages of elastin, the samples had been wrongly labeled. The general run of these results supports the common belief that the inner round is tenderer than the outer round, and the custom in some markets of charging more for the former.

To the biologist it may not appear remarkable nor even unexpected, that the amount of fat on an animal carcass or its distribution among the different parts of the carcass, or even the appearance of the lean itself, should bear no relation to the toughness of the lean insofar as this is determined by the connective tissue contained in it. However, to the practical man concerned with the selecting and grading of meat this lack of agreement may seem incomprehensible, since he has become so accustomed to the belief that these characters of the carcass are reliable indices of the palatability of the meat. Nevertheless, this belief is not, insofar as the authors are aware, supported by experimental data. The data here reported, do not lend support to the belief that ordinary methods of judg-

ing meat give reliable information concerning toughness of the lean, insofar as this is determined by its content of connective tissue.

Summary

1. An improved method of determining the collagen and elastin in uncooked animal tissues is described, and its accuracy is measured and discussed. The results of its application to beef muscle from carcasses of definite antecedents and description are given.

2. No constant and significant differences in connective tissue content were found between heifer and steer beef.

3. Among different retail cuts of beef examined, the eye-muscle of the rib possessed for practically all calves examined the lowest content of collagen, expressed as collagen nitrogen in per cent of total nitrogen. The tenderloin was only slightly higher. The round, porterhouse and sirloin were next in order and were indistinguishable from each other in this respect. The chuck ribs and the navel contained consistently larger percentages of collagen nitrogen than the more expensive cuts, but the fore shanks of all calves contained the highest, the collagen nitrogen of this cut averaging 23 per cent of the total nitrogen of the lean. The outer round generally contained more collagen than the inner round.

4. The percentage of elastin in muscle is only a small, in most cases an insignificant, fraction of the percentage of collagen. The distribution of elastin among the different retail cuts of meat differs from the distribution of collagen, but appears to be as consistent and as significant.

5. Age does not seem to have a great effect upon the connective tisse content of muscle meat, nor a consistent effect among the different muscles of the carcass. However, much of the data obtained on this point were so irregular as to preclude any definite conclusions.

6. No relation at all was noted between the ordinary market grading of beef carcasses and the connective tissue content of the lean of the rib (eye) or of the round. Insofar as the tenderness of meat is related to its content of connective tissue, the results obtained in this investigation lend no support to the belief that the appearance, texture and firmness of its lean gives reliable information concerning its tenderness.

7. No obvious relation was found between the grading of beef carcasses and the fat content of the lean of selected samples.

The authors wish to express their appreciation to Professor Sleeter Bull for his assistance in securing the samples of meat used in this investigation and the carcass gradings quoted.

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IMPROVED RATE OF GROWTH OF STOCK ALBINO RATS

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WITHIN recent years several investigators have called attention to the heightened growth rates that are now attainable with albino rats. It is not unusual in more than one laboratory today for the average rat of a given age in the colony to be beyond the maximum weight for that age reported in such standard tables as are given in Donaldson's book, *The Rat*. Greenman and Duhring (1) in 1923 at the Wistar Institute reported much better growth, with greater weight at maturity, than previously had been obtained there. Still more rapid growth has been reported by Macy, Outhouse, Long and Graham (2). These accelerated rates of development have resulted, seemingly, from improved feeding methods, although the continuous selection of thrifty animals for breeders is a factor to be considered in any interpretation of this phenomenon. By the inclusion of lettuce or liver as accessories to their stock ration Osborne and Mendel (3) succeeded in obtaining unusually large and flourishing animals.

The colony in this laboratory was started in 1927 from stock of the Connecticut Agricultural Experiment Station and it has been found that the best growing animals are quite comparable in size to the selected groups of Osborne and Mendel (3) and Mendel and Cannon (4). At 30 days of age our male rats average 66 grams, which is 24 grams heavier than the rats of the same age as reported by Greenman and Duhring, and about 16 grams heavier than the average of Dr. Macy's colony. The females also are heavier, the animals 30 days of age averaging 63 grams, as compared to 39 grams for Greenman and Duhring's rats and 50 grams for those of Macy, Outhouse, Long and Graham. The average weight of the male rats 200 days old is 423 grams, or about 123 grams more than the males of that age as reported by Greenman and Duhring, and by extrapolation of the growth curve of Dr. Macy's colony, is about 48 grams heavier than her average value. The females are also heavier throughout their life span than most stock rats reported in the literature, but the differences are not so marked as with the males.

For the animals herein described the commonly accepted standards of growth are scarcely significant. A rat that would be "subnormal" according to the new standard, so far as growth in body weight is concerned, might still be well above the normal weight of former so-called standards. Moreover, attempts are now being made to compare rations of different natural foods with a view of producing either optimal nutrition or of comparing substances added to a complete mixture of the known dietary essentials (5). The differences in the growth rates on the various diets in such studies may not amount to very many grams, and better standard curves for comparison would aid in their interpretation. Because of the usefulness of growth curves in all experimental work in nutrition, and as data for the interpretation of the nature of growth itself, we have determined the normal rate of growth for our colony under the present conditions and with the present stock diet.

METHODS OF FEEDING

The ration fed to our stock animals is essentially Sherman's normal diet B (6), consisting of $\frac{2}{3}$ ground whole wheat, $\frac{1}{3}$ dried whole milk, plus sodium chloride to the extent of 2 per cent of the weight of the wheat. We have found it advantageous to replace half of the added sodium chloride with an equal weight of calcium carbonate. The calcium to phosphorus ratio in the unmodified Sherman diet is only 0.65, but with the addition of calcium carbonate as mentioned, so that it makes up 0.658 per cent of the entire mixture, the Ca to P ratio is 1.16. The recent metabolism experiments of Haag and Palmer (7) indicate that the ratio of Ca to P retained by the growing rat is about 1.2. Shohl, Bennett and Weed (8), using animals from the colony herein discussed, found that the ratio of retention in their best growing rats was 1.33. It is interesting to note that the Ca to P ratio in cow's milk is 1.3. Milk is also a potentially alkaline food, as estimated by the method of Sherman and Gettler (9) from published analyses. The Sherman diet B without calcium carbonate is equivalent to about 9 cc. 0.1 N acid per 100 grams of food. With the added calcium carbonate the diet becomes equivalent to 123 cc. of 0.1 N alkali per 100 grams. It is highly probable that the improved growth observed in our colony is due in large part to the more favorable relationship between calcium and phosphorus as well as to the alteration in the potential reaction of the diet.

In addition to the above mixture all stock rats are given fresh lettuce daily. Lactating rats receive also 9 grams of dried yeast per week, in

order to meet the added requirements for the vitamin B complex during the nursing period.

Female rats are not bred until about three months of age. Two weeks resting period is allowed females between each weaning and remating, and no female is bred more than three times. The litters are reduced to eight young at birth. The young rats open their eyes about the sixteenth day, and begin to eat a little of the stock rations supplied to their mothers. On the twenty-first day the young are weaned. With this regimen healthy and vigorous stock always have been obtained.

GROWTH DATA

For obtaining growth data every male rat and unmated female in the stock colony were weighed at 10 day intervals. The age intervals used in plotting the growth data were 21 days, 23 days (including 22 to 24 days), 27 days (including 25 to 29 days) and so on in 5-day intervals to 49 days of age. Thereafter the age intervals were 10 days in length. The average age was taken as the median day of each interval. We believe the ages were sufficiently scattered and the number of animals large enough to justify this procedure. Nearly 1500 weighings were made of male rats and over 500 of females. These animals were born for the most part during the late summer and early fall of 1927.

In order to ascertain whether seasonal or other variations might affect the data so obtained, the validity of the method was tested by a smaller group consisting of four litters of animals, two born in January and two in April. These litters were treated in exactly the same manner as all of the stock rats in the colony. They were segregated into groups according to sex at thirty-five days of age. The weights of both males and females were determined at intervals of three to five days between 21 and 90 days of age. The observations thus extended through the periods of wide individual variation that are found during adolescence and puberty, as ascertained from the data on the coefficient of variation for the stock colony. The growth curves of both males and females of these test litters closely duplicated the average for the colony. The rats born in the winter grew at a slightly slower rate than the colony curve, but were well within the range of the standard deviation of the colony. Those rats born in the spring grew at an indistinguishable rate from the stock colony until 60 days of age, and then grew at a little faster rate but as with the winter litters, the average for each age was well within the range of the standard deviation of the colony. This corroborates the findings of Hanson and

Heys (10), who showed that slight seasonal variations in the growth rate of the albino rat are to be expected.

The data are presented in Tables I and II, for males and females respectively. The average weight of the animals in each age interval is given, as well as the maximum and minimum weights observed, and the number

TABLE I

THE RATE OF GROWTH OF MALE ALBINO RATS ON STOCK DIET

Age	Average Weight	Minimum Weight	Maximum Weight	Number of Rats	Standard Deviation of Mean	Coefficient of Variation
days	grams	grams	grams		grams	per cent
21	40	24	52	88	5.06	12.71
23	46	35	56	53	5.13	11.25
27	63	45	83	53	9.39	14.95
32	82	50	117	75	15.52	18.88
37	110	84	147	42	15.00	13.64
42	126	90	153	67	14.91	11.83
47	157	112	205	52	22.39	14.26
55	188	123	278	104	35.27	13.44
65	221	122	313	95	34.59	15.61
75	268	205	320	73	26.94	10.05
85	285	186	355	46	31.62	11.10
95	317	233	387	47	37.52	11.84
105	327	292	405	16	34.50	10.55
115	330	250	432	52	33.58	10.18
125	336	250	397	68	33.91	10.09
135	352	255	415	67	37.88	10.76
145	370	258	443	68	36.08	9.75
155	384	295	445	67	37.28	9.71
165	391	314	467	71	48.21	12.33
175	400	315	497	66	40.99	10.25
185	420	332	535	32	48.50	11.55
195	438	300	543	31	55.86	13.69
205	424	327	556	37	49.84	11.75
215	430	322	553	36	49.20	11.44
225	424	360	468	19	30.47	7.19
235	448	343	556	29	49.91	11.14

The age groups were 21 days, 22 to 24 days inclusive, average 23; 25 to 29 inclusive, average 27; and so on to 45 to 49 days. Then the groups were in ten-day periods, 50 to 59 days, average 55 etc.

of rats. The observations were treated by accepted statistical methods and the standard deviations and the coefficients of variation calculated. The formula used for the standard deviation was:

$$\sigma = \sqrt{\frac{\Sigma d^2}{N}}$$

in which σ = standard deviation of the average
Σd^2 = sum of the deviations from the average, squared
N = number of observations.

TABLE II

THE RATE OF GROWTH OF FEMALE ALBINO RATS ON STOCK DIET

Age	Average Weight	Minimum Weight	Maximum Weight	Number of Rats	Standard Deviation of Mean	Coefficient of Variation
days	grams	grams	grams		grams	per cent
21	39	25	47	88	4.29	10.91
23	42	34	52	19	5.46	12.87
27	60	43	80	11	11.49	19.19
32	74	52	90	19	11.21	15.25
37	103	82	127	11	15.96	15.49
42	110	84	143	17	17.71	16.10
47	137	120	158	14	12.78	9.33
55	150	108	205	38	23.07	15.38
65	162	115	207	28	25.63	15.82
75	178	133	233	32	25.92	14.56
85	189	135	221	28	20.90	11.06
95	201	175	247	24	27.45	13.65
105	212	190	246	17	15.49	7.31
115	219	185	268	21	21.16	9.66
125	221	187	278	39	21.43	9.70
135	221	190	250	32	15.44	6.99
145	229	197	257	31	18.30	7.99
155	234	203	265	25	18.39	7.86
165	236	204	265	30	16.93	7.18
175	238	205	272	27	17.80	7.48
185	242	213	270	27	16.84	6.96
195	249	213	273	13	16.24	6.52
205	250	213	273	18	15.68	6.27
215	250	225	270	10	15.35	6.14
225	248	222	283	12	19.60	7.90
235	248	222	265	12	24.01	5.65

For the coefficient of variation the following formula was used:

$$\text{C. V.} = \frac{\sigma \times 100}{\text{Average}}$$

in which C. V. = coefficient of variation
σ = standard deviation.

The standard deviation gives a measure of the absolute variability of the observed weights, and the coefficient of variation, a relative measure

of the variations as compared to the magnitude of the average weights. Thus, from the table, at 42 days of age the average weight of the male rats is 126 grams and the standard deviation is 14.91 grams. At 95 days of age the average weight is seen to be 317 grams and the standard deviation is 37.52 grams. But the coefficient of variation for both ages is the same, namely, 11.8 per cent, which indicates that the variability of the data in these two groups is exactly the same. It is also evident from the tables

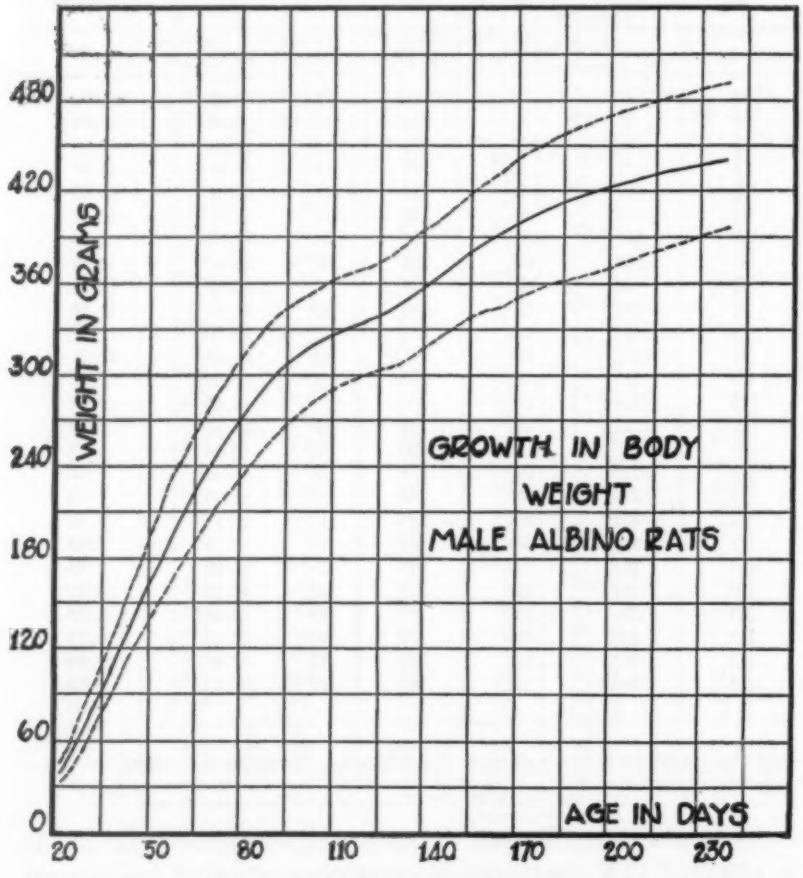

CHART 1. The rate of growth of male albino rats. The dotted lines represent the smoothed standard deviation curves, the vertical distance from each dotted line to the average curve being equal to the standard deviation. Within this area enclosed by the dotted curves are included about two-thirds of the rats of the colony.

that the variability of the weights is greater for young rats than for old, particularly with the females, where the number of observations is comparatively few. But after puberty the weights are more uniform, and with the females, remarkably so.

The smoothed growth curves are represented in Charts 1 and 2 by the heavy lines. Following the suggestion of Macy *et al.* (2), the standard deviations are represented on each chart by the dotted curves on each side of the average. The vertical distance between each dotted line and the average curve is equal to the standard deviation. It has been shown

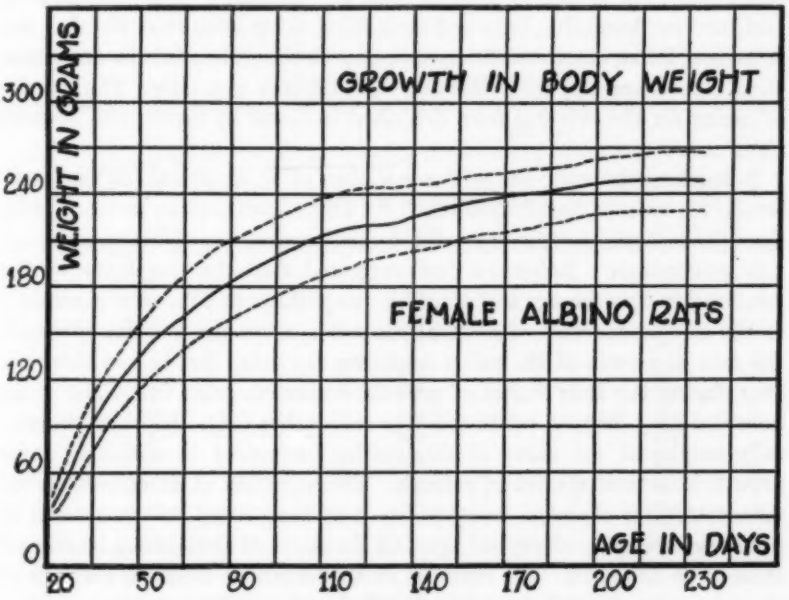

CHART 2. The rate of growth of female albino rats. The dotted lines represent the curves of the standard deviations plotted plus and minus from the average.

that in a symmetrical distribution curve, 68.26 per cent of all the observations lie between the mean and the standard deviation measured plus and minus from the mean (11). Therefore, the observations on about $\frac{2}{3}$ of all stock rats in this study should be included within the area blocked out by the dotted lines. For example, at 215 days of age the average weight of the male rats is 430 grams, as read from the chart, or from Table I. At this age, the weights of all the rats measured fell between the minimum and maximum weights of 322 and 553 grams. The weights of $\frac{2}{3}$ of the total

number of rats weighed, or 24 of the 36 animals in this group, fell between 381 grams and 479 grams, these figures being obtained by measuring off the standard deviation above and below the average. In addition, the chances are 2 to 1 that the average weight of any other group of males, 215 days old will fall within these same limits.

In view of the uniform and exceptionally rapid growth of the rats of this colony other workers in the field of nutrition might be led to attempt to apply uncritically these newer growth curves to their animals. We wish to emphasize the fact that environment plays a large part in enabling the hereditary growth factors to exert their full influence. The conditions of temperature, humidity, light and sanitation, quite aside from the diet, are of highest importance and we believe that in the colony herein described, these factors are well controlled and exceptionally favorable. The scheme of caring for the rats has been described in detail by Smith and Mendel (12).

Robertson has made use of the equation of an autocatalytic monomolecular reaction to describe growth (13). This equation has a rational basis, since the essential feature of growth is the chemical transformation of food into protoplasm. Robertson has suggested that the simplicity of the relationship between age and weight is due perhaps to a "master reaction," or the slowest reaction of a metabolic series, upon the velocity of which the rate of growth of the entire organism depends. Brody has observed that during the later stages of growth, successive gains in weight of an animal bear a definite relationship to each other (14). He then successfully employed the curve of diminishing increment in analyzing later growth in several species of animals. The equation of an autocatalyzed monomolecular chemical reaction has here been fitted to the growth of our male rats up to 65 days of age, and the curve of diminishing increment from 65 to 235 days. The curve of an autocatalytic chemical reaction of the first order as applied to growth follows this equation:

$$\log \frac{W}{A - W} = k\,(t - t_1)$$

in which W = the weight at any time t, in grams

A = weight which animal approaches asymptotically as the cycle of growth involved approaches completion

k = the velocity constant of growth

t = age, in days from birth

t_1 = age at which the cycle is half completed,

when $W = \frac{A}{2}$

For our male rats between the ages of 21 and 65 days this equation becomes, when calculated by the method of Brody (15) with the aid of Robertson's tables (13),

$$\log \frac{W}{300 - W} = .030\,(t - 47)$$

The agreement between the calculated and observed values as given in Table III, is only fairly close, perhaps because it was assumed that the growth made in any previous cycle to the one under consideration was of negligible magnitude.

TABLE III

WEIGHT OF MALE ALBINO RATS AS CALCULATED BY THE EQUATION FOR AN AUTOCATALYZED, MONOMOLECULAR CHEMICAL REACTION

Age from Birth	Weight	
	Calculated	From Chart
days	grams	grams
21	43	40
23	48	45
27	60	60
32	79	80
37	100	105
42	125	129
47	150	150
55	190	184
65	233	223
75	262	255
85	280	284

The equation for the curve of diminishing increment is

$$W = A - Be^{-k(t+22)}$$

in which W = the weight at any time t, in grams
A = the mature weight of the animal which is approached asymptotically as t approaches infinity
B = a constant upon which the gain in weight is dependent
e = the base of natural logarithms
k = a constant indicating the rate of decline in growth
t = the age in days from birth
22 = the number of days of gestation

Solving by the graphic method of Brody gives:

$$W = 466 - 725e^{-.0127(t+22)}$$

The calculated and observed weights as read from the growth curve, from 65 to 235 days of age, are given in Table IV. The agreement is quite close especially for the beginning and end of the period considered. The greatest discrepancy occurs in the neighborhood of 125 days that may, perhaps, be due to some unknown environmental change in the rat room.

TABLE IV

WEIGHTS OF MALE ALBINO RATS AS CALCULATED BY THE FORMULA FOR THE CURVE OF DIMINISHING INCREMENT

Age from Birth	Weight	
	Calculated	From Chart
days	grams	grams
65	226	223
70	241	240
75	255	255
80	268	270
85	279	284
95	302	307
105	322	321
115	339	331
125	358	338
135	368	350
145	379	364
155	389	379
165	399	392
175	407	404
185	413	413
195	420	420
205	425	425
215	430	430
225	435	434
235	438	438

A more thorough algebraic formulation of the growth curves will be undertaken when more data are gathered on the weights of our rats, both male and female, before weaning and also before birth. From the equation as given, the average mature weight which the male rats approach is 466 grams. By substitution in the equation, the rats will have attained 98 per cent of this weight, or 456 grams, when they reach 316 days of age.

It is remarkable that the curve of diminishing increment is apparently

applicable to so long a portion of the growth curve of these rats. Titus and Jull (16) have speculated that the more nearly a perfect condition of nutrition is approached the more nearly will the whole of the growth curve approach the curve of diminishing increment. But in the case of the albino rat it may be that in addition to a more perfect nutritive condition, we have morphological changes that are tending toward the production of a new species. Indeed, cytological evidence supporting this view has recently been advanced by Swezy (17).

The foregoing data together with the voluminous statistics already available on the biometry of the white rat not only may be an evidence of evolution in this species but also may indicate the care required in evaluating data obtained in experiments involving the use of the albino rat.

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Editorial Review

NUTRITIONAL SIGNIFICANCE OF OUR PRESENT KNOWLEDGE OF THE MULTIPLE NATURE OF VITAMIN B.

IT IS now well established that what in the past has been called vitamin B is not an entity; but comprises more than one nutritionally essential substance.

The need of an antineuritic factor in both mammalian and avian nutrition has been recognized from the very beginning of our knowledge of vitamins. For about a dozen years it was generally thought that this antineuritic factor might perhaps be identical with the water-soluble growth-essential which McCollum had named "water-soluble B." At no time was this generally regarded as proven; but in view of what seemed a reasonable possibility, and in the absence of convincing evidence to the contrary, most writers, tentatively and in the interest of simplicity and convenience, followed the recommendation of Drummond and used the term *vitamin B* in place of both the previous terms *antineuritic vitamine* and *water-soluble B*.

Various writers from time to time, and notably Mitchell (1919) in a critical discussion, called attention to the fact that not all of the published evidence was consistent with this simplifying assumption; but only slowly (slowly at least as things go in the rapidly moving history of the vitamins!) did there accumulate such a preponderance of experimental evidence for the multiple nature of vitamin B as to command the universal acceptance of the students of this problem.

It is not the purpose of this review to discuss the question of priority of discovery of the multiple nature of vitamin B, nor to set forth the results of the purely chemical work which has been done in the attempt to isolate the antineuritic vitamin. Our present endeavor is rather to indicate, in as brief and non-technical a manner as possible, the nutritional significance of present knowledge (the time of writing being the summer of 1928).

The publications here referred to are not offered as a complete bibliography of the subject, and as we are dealing with the present status of a point of view and not with the history of the discovery of facts or the promulgation of ideas, the reader is earnestly requested not to read into this brief and necessarily sketchy outline any implication of assignment

of credit for priority or of attempt to represent the precise view of any given investigator at any particular time.

Emmett and Luros, in 1920, reported the results of a systematic investigation in which identical food materials were fed for the prevention of polyneuritis in pigeons and for the support of growth in rats. Furthermore, these comparative feeding experiments were repeated with portions of the same food materials which had been heated at different temperatures and under different conditions. They concluded that the antineuritic vitamin seemed to be less stable to heat and alkali than the rat-growth-promoting vitamin.

Funk and Dubin, in 1921, recorded very briefly some experiments upon fractional adsorption which led them to believe that autolyzed yeast contained two water-soluble vitamins, of which the antineuritic was the more readily adsorbed by fuller's earth.

Levene and Muhlfeld (1923) found evidence of the multiple nature of vitamin B in the fact that their experiments upon the concentration of this factor resulted in altering the relative antineuritic and growth-promoting activities of their materials.

Kinnersley and Peters (1925), who also were working primarily from the point of view of isolation of the antineuritic vitamin, reported the separation of a highly antineuritic substance which did not cause increase in the weight of pigeons after cure of the polyneuritic symptoms. This was interpreted as supporting the view of the multiple nature of vitamin B.

In the same year, this view was discussed tentatively by McCollum, Simmonds and Becker (1925).

In 1926, Hauge and Carrick published further evidence of the fact that antineuritic and growth-promoting potencies do not always run parallel, and in the same year the subject was greatly clarified by three contributions from the United States Public Health Service and two from the laboratory of McCollum, all of which emphasized the important fact that the growth of rats (and there is no reason to doubt that this is true of mammals generally) requires *both* the antineuritic vitamin and another, more heat-stable, substance which along with the antineuritic substance had hitherto been covered by the term vitamin B.

In the course of an investigation begun as a study of the influence of dietary deficiencies upon experimental tuberculosis in the albino rat, Smith and Hendrick (1926) found that a diet containing 40 per cent of rolled oats, with adequate amounts of casein, salts, and vitamin-A-containing fats, was not adequate to support normal growth of young rats; but became so when supplemented by yeast which had been autoclaved for 6 hours

at 15 pounds steam pressure, a procedure believed to destroy the antineuritic vitamin of the yeast. And, furthermore, that neither autoclaved yeast alone nor Seidell vitamin-B picrate alone would meet the growth-promoting vitamin B requirement of rats; but that they supplemented each other in such a way that together they met this vitamin B requirement. This work of Smith and Hendrick was immediately confirmed by Seidell (1926) who, as the result of experiments with both rats and pigeons, suggested that rats (and probably other mammals) need both the antineuritic substance and the other, more heat-stable, factor of vitamin B, while pigeons need only the antineuritic vitamin; and for this reason he recommended that the rat be used for determinations of vitamin B and the pigeon, on account of its simpler needs, for studies of the antineuritic vitamin (see, in this connection, the work of Williams and Waterman, cited below).

Closely following the contribution of Smith and Hendrick, appeared the independent and practically simultaneous work of Goldberger, Wheeler, Lillie and Rogers (1926) who found a similar supplementary relationship, in meeting the growth requirements of young rats, between autoclaved yeast (as the source of the heat-stable factor) and an alcohol-extract of corn meal (as the source of the antineuritic substance). This work was discussed in its bearings upon other investigations of Goldberger and his associates upon human pellagra and the more or less analogous condition known as black-tongue in dogs. Goldberger and Tanner (1925) had postulated the existence of a previously unrecognized dietary factor concerned in the prevention of pellagra (tentatively designated as the "P-P factor" or "pellagra-preventing vitamin"), and this was now held to be identical with the heat-stable factor of vitamin B, the rats whose rations lacked this factor being described by Goldberger, Wheeler, Lillie and Rogers as developing a pellagra-like condition after about nine weeks of this dietary deprivation.

Meanwhile Laird (1926), in McCollum's laboratory, found in the testing of numerous extracts obtained from natural foods by means of different (usually acidulated) solvents, repeated instances in which antineuritic and growth-promoting potencies did not run parallel, and some cases in which growth was not promoted by either of two extracts but was induced by a mixture of them. The mutual bearings of this work and that of the U. S. Public Health Service above mentioned were fully discussed by McCollum (1927) in a communication of August 1926, which on account of unavoidable delays in publication did not appear in print until the following year.

Salmon (1927), working with soy beans and velvet beans and with the leaves of velvet beans and of rape, found that the seeds were relatively more potent for the prevention of polyneuritis and the leaves for the promotion of growth. He also described a partial separation of the antineuritic from the other growth-promoting factor, chiefly by extraction with alcohol and adsorption upon fuller's earth. The alcohol-extract, in the form of a water solution, was treated for the removal of ether-soluble substances and of substances precipitable by lead acetate, before the treatment with fuller's earth. The leaves were extracted with acidulated water instead of alcohol. (The scope of the present review does not permit of any attempt at full description of methods.) The continuation of this work has been described in a later paper by Salmon, Guerrant and Hays (1928), who report that fuller's earth adsorbs both factors; but, under certain conditions, the antineuritic more completely. They describe the preparation, from velvet bean leaves, of a fraction practically freed from the antineuritic but still rich in the other factor; and promise to report, in a later paper, upon a detailed study of the effect of hydrogen ion concentration upon the adsorption of the antineuritic vitamin.

Meanwhile Chick and Roscoe (1927) reported a series of experiments in which various extracts and preparations of yeast and of wheat embryo were fed singly and in combination with results which demonstrated still further the existence in the vitamin B complex of more than one factor essential to growth; and Hassan and Drummond (1927), in the course of a study of the possible relation between vitamin B and protein metabolism, independently obtained evidence of the presence of two growth-promoting factors in what had been known as vitamin B.

The work of Palmer and Kennedy (1927–1928), while chiefly presented from a somewhat different angle, seems also to furnish a large amount of evidence of the multiple nature of vitamin B. Evans and Burr (1928) have recently furnished a clear-cut demonstration.

Williams and Waterman (1927, 1928) hold that vitamin B is of a tripartite nature, involving the antineuritic factor required by both rats and pigeons, a second factor required by rats but not by pigeons, and a third substance needed by pigeons but not by rats. In the present review only the first two of these factors are considered.

Evidence of a more quantitative nature was obtained by Sherman and Axtmayer (1927), who measured, by means of the averages of large numbers of quantitatively conducted feeding experiments, the relative richness, in the two factors required by rats, of whole wheat on the one hand and of milk or of autoclaved yeast on the other. That heating in the

autoclave may destroy the antineuritic potency of yeast while leaving it still a good source of the other factor had been shown by previous workers, as explained above. The present investigation established a marked supplementary relationship between wheat and milk, wheat being relatively richer in the antineuritic, and milk relatively richer in the more heat-stable factor. This work thus furnished a basis for the quantitative reinvestigation and reinterpretation of the previously recorded "vitamin B values" of foods.

Hunt (1928) found corn (maize) to be very similar to wheat in its content of the two factors required for growth of rats.

Eddy (1927, 1928) found that the previously reported low vitamin B value of cooked or canned spinach is due to paucity of the antineuritic factor in a material relatively rich in the more heat-stable vitamin; and also that bananas are relatively richer in the heat-stable than in the antineuritic factor.

Hogan and Hunter (1928) have very recently reported that just as by heating the antineuritic factor can be more or less selectively destroyed, so the more heat-stable factor may be selectively destroyed by exposure to ultraviolet irradiation.

There has also been much recent activity in the investigation of the purely chemical nature of the antineuritic vitamin. The discussion of these investigations will, however, not be undertaken here; for this line of research, having already yielded a voluminous literature but not yet a clear concensus of opinion, and being still extremely active, may well be the subject of a separate review at a later time.

Thus the multiple nature of vitamin B is established by an abundance of evidence, and evidence of several different kinds.

It no longer appears profitable to perform experiments or to publish papers in which vitamin B (in the sense in which the term has hitherto been used) is treated as an entity.

But previous experiments of this kind are by no means entirely without value. Without proper interpretation they may be misleading, and therefore worse than useless; but if properly interpreted they may still furnish us information of real importance, especially as to the nutritive values of foods. Most such experiments have been made by means of rats which need for health, for growth, and even for the maintenance of their body-weights, both the antineuritic and the more heat-stable substance.

Foods which, by means of rat feeding experiments, have been shown to be good sources of vitamin B as previously understood are, therefore,

now seen to be good sources of both the antineuritic substance and the more heat-stable vitamin.

If, on the other hand, the food, when tested for undifferentiated vitamin B, has not given good results, it is not necessarily poor in both of these essentials, but may conceivably be a good source of one or the other of them, and should therefore, be tested for each separately.

Moreover a food which is a good source of both may still be markedly richer in one than the other, as the investigations above cited have shown to be true in the case of milk, and of typical seeds and green leaves.

Milk and green leaves, good sources of both these factors, are relatively richer in the more heat-stable factor. Whole grain cereals and legumes, fair-to-good sources of both factors, are relatively richer in the antineuritic vitamin. Thus both milk and green leaves here show a valuable supplementary relationship to seeds with respect to vitamin B, as had previously been shown to be the case with respect to vitamin A.

The development of methods for the separate quantitative measurement of values of foods as sources of the two separate factors is now well advanced, and, in view of the interest already displayed in vitamin B as a factor in food values, it is to be hoped that the quantitative study of the two factors of vitamin B now recognized as essential to mammalian nutrition will be taken up promptly and pursued actively by a number of laboratories, so that we shall before long know the relative richness, in each of these factors, of all the more important foods.

In dealing with the results of quantitative determinations of (old style) vitamin B values of foods, we are at present in a situation somewhat analogous, from the nutritional point of view, to that in which we have often found ourselves in the past when nutritive efficiencies of the proteins of foods have been measured by means of careful feeding trials in advance of the determination as to which of the nutritionally essential amino acids was the limiting factor in each case. Unless the limiting factor is known to be the same in both, any two foods may prove to have supplementary relations of great nutritional importance, as wheat and milk are now known to have both as regards their proteins, and as regards their vitamin B values.

The exact relation of the heat-stable factor to the prevention of pellagra is a problem of great scientific and practical interest which, however, appears to admit of only tentative treatment at this time. The observation of Goldberger and his coworkers that this substance prevents the develop-

ment of certain more or less "pellagra-like" symptoms in the rat, has been abundantly confirmed.

Meantime, however, Underhill and Mendel (1928) have shown that pellagra-like symptoms in the dog can be prevented by what appears to be an entirely different substance—a fat-soluble factor occurring abundantly in butterfat and carrots and apparently identical with (at any rate experimentally replaceable by) carotin.

Even if one be prepared to put aside completely the earlier explanations of nutritional prevention of pellagra, which it might seem somewhat hasty to do in view of the experimental evidence which had been offered in their support, there still appear from the most recent work to be two substances of different physical properties and no probable chemical relationship, both potent in the prevention of pellagra-like symptoms.

Moreover, as the reports now stand (August 1928) there are differences between certain of the empirical findings of the two groups of investigators, which until clarified by further experimentation would seem to preclude any satisfactory attempt at this time to combine all the offered evidence under one consistent interpretation. Further discussion of the pellagra problem is therefore postponed.

The discussion of the evidence of the multiple nature of vitamin B has involved emphasis upon the fact that heating in the autoclave destroys the antineuritic vitamin but not the other factor, and consequently frequent references to the latter as the more heat-stable factor have been made. This fact might readily tend to give an exaggerated impression of the susceptibility of the antineuritic vitamin to destruction upon heating.

Something should therefore be said of the quantitative studies of the stability or lability of the antineuritic substance, because of the nutritional significance of this property in relation to vitamin values of foods which have been heated or stored under different conditions.

In the dry state, at practically neutral reaction, the antineuritic vitamin is quite stable to heating at 100°, showing no measurable destruction or inactivation after 48 hours of such heating; whereas the same material heated in water for 6 hours at 100° showed a slight diminution of potency (Sherman and Spohn, 1923). Although this work was done before the differentiation of the vitamin B complex, it is certain that it was the antineuritic substance whose stability was measured, both because the so-called antipellagric vitamin is so much more stable and because the work of Sherman and Axtmayer has shown plainly that cows' milk is richer in this vitamin than in the antineuritic substance, so that the latter must

have been the limiting factor in the rat growth experiments of Sherman and Spohn.

In other experiments (Sherman and Grose, 1923; Sherman and Burton, 1926) the rate of destruction of "vitamin B" was measured at different temperatures at the normal acidity of tomato juice; and later for different hydrogen ion activities at a fixed temperature of heating of 100°. While it has not yet been shown whether the antineuritic substance is the limiting factor of the vitamin B complex of tomato juice, yet in view of the much greater stability of the other vitamin, it seems practically certain that the experiments here cited may be taken as affording at least approximate measures of the rate of destruction or inactivation of the antineuritic vitamin under the conditions described.

Such reinterpretations into terms of the newly recognized factors will doubtless prove feasible with much of the previously recorded work relating to the vitamin B complex, especially when the work has been of a quantitative nature.

With still greater interest may we anticipate new experimental work conducted separately upon the two (or more) essential substances of the vitamin B complex. Both are doubtless of much nutritional significance.

H.C.S.

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